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Comments on Preble's Meadow Jumping Mouse Delisting Proposal

(Listed in order received. Dates are those on comments.)

Reopened Comment Period

29. 2/6/06 Mark Lusch, Cheyenne, WY

30. 2/18/06 Tom and Mary Ann Cunningham, Green Mountain Falls, CO

31. 2/18/06 Bruce Roberts, Monument CO

32. 2/20/06 Mitchell Baldwin

33. 2/21/06 Oliver A. Richardson

34. 2/22/06 Robert B. Hoff, Colorado Springs, CO (see 1 and 6 above)

35. 2/22/06 Colleen Miller

36. 2/21/06 Linda Samelson, Colorado Springs, CO

37. 2/26/06 Jennifer K. Frey, Frey Biological Research, Radium Springs, NM

38. 2/25/06 Nick Ordon, Falcon, CO

39. 3/1/06 Unsigned, Colorado Springs, CO

40. 3/9/06 Leslie Barstow, Golden, CO

41. 3/9/06 Peter Bray, Portland, OR

42. 3/9/06 Donna Miller, Golden, CO

43. 3/13/06 Daryl E. Mergen, Colorado Springs, CO

44. 3/31/06 Ronald W. Opsahl, Staff Attorney, Mountain States Legal Foundation, Lakewood, CO (See 7 above)

45. 3/31/06 C. J. Rapp, Littleton, CO

46. 4/4/06 Ken Faux, Greenwood Village, CO (see 18 above)

47. 3/31/06 Ken Hamilton, Executive Vice President, Wyoming Farm Bureau Federation, Laramie, WY

48. 3/31/06 Renee C. Taylor, Environmental Coordinator, True Ranches, LLC, Casper, WY (see 12 above)

49. 4/13/06 Robert E. Arlen, Science Faculty, University of Phoenix, Casper, WY

50. 4/17/06 Sandra A. Eddy, Aurora, CO

51. 4/18/06 Kent Holsinger, Hale Friesen, LLP, Denver, CO. On behalf of Colorado Water Conservation and Development

52. 4/28/06 Robert A, Schorr, Zoologist, Colorado Natural Heritage Program, Colorado State University, Fort Collins, CO

53. 4/28/06 Eric Hallerman, Professor, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA

54. 5/11/06 Sacha Vignieri, Center for Study of Evolution, University of Sussex, Brighton, UK

55. 5/15/06 Jonathan Dowling, Assistant Vice President, Wyoming Contractors Association, Cheyenne, WY

56. 5/1/06 Sallie Clark, Chair, Board of County Commissioners of El Paso County, Colorado Springs, CO

57. 5/16/06 Sylvia M. Fallon, Conservation Genetics Fellow, Natural Resources Defense Council

58. 5/17/06 Don Britton, Manager, Wheatland Irrigation District, Wheatland, WY

59. 5/17/06 Dale Moore

60. 5/18/06 Carron Meaney (Meaney and Co.; Reasearch Associate, DMNS; Curator Adjoint, University of Colorado Museum), Thomas Ryon (Wildlife Biologist and Certified Ecologist), Mark Bakeman (President, Ensight Technical Services Inc.) and Anne Ruggles (Bear Canyon Consulting), CO

61. 5/18/06 Tina Comerford, Wheaton, IL

62. 5/17/06 Niel A. "Mick" McMurry, Shareholder, Sybille Ranch LLC, Cheyenne, WY

63. 5/18/06 Rob Roy Ramey, II, Nederland, CO

64. 5/18/06 Jim Magagna, Executive Vice President, Wyoming Stock Growers Association, Cheyenne, WY

65. 5/18/06 Erin Robertson, Staff Biologist, Center for Native Ecosystems, Denver CO. On behalf of: Jeremy Nichols, Conservation Director, Biodiversity Conservation Alliance, Denver, CO and Nicole Rosario, Conservation Director, Forest Guardians, Santa Fe, NM (See 23 above)

66. 5/18/06 Patrick J. Crank, Attorney General, State of Wyoming, Cheyenne, WY

67. 5/19/06 Cheryl Matthews, Director, Douglas County Division of Open Space and Natural Resources, Castle Rock, CO (See 19 above)

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04/28/2006 11:37 AM

To <FW6_PMJM@fws.gov>
cc
bcc
Subject Comment on delisting of Preble's meadow jumping mouse

Department of Fisheries and Wildlife Sciences

Virginia Polytechnic Institute and State University

Blacksburg, VA 24061-0321

April 28, 2006

Susan Linner, Field Supervisor
Colorado Field Office, Ecological Services
P.O. box 25486
Denver Federal Center
Denver, CO 80228

Dear Ms. Linner,

The U.S. Fish and Wildlife Service on February 17 reopened the comment period for the proposed delisting of the Preble's meadow jumping mouse, *Zapus hudsonius preblei* . This reopening follows the reporting of very different conclusions by two recent taxonomic studies. On the one hand, Ramey and colleagues concluded that recognition of *Z.h. preblei* was not justified, and that *Z.h. preblei* should be merged with *Z.h. camprestris* and *Z h. intermedius* . On the other hand, King and colleagues concluded that subspecific recognition of *Z.h. preblei* is justified. I welcome the opportunity to submit comments regarding the relative strengths of the respective studies and the defensibility of their conclusions.

I offer my comments from the point of view of a population geneticist. I have been involved in population genetics research for over 25 years, and have published over 75 peer-reviewed papers and a textbook on population genetics. I am a former president of the Genetics Section of the American Fisheries Society. I have shared my expertise with numerous public agencies, including the U.S. Department of Agriculture, the Food and Drug Administration, the National Research Council, and the Food and Agriculture Organization and World Health Organization of the United Nations.

Comments on Ramey et al. Ramey et al. examined variability in cranial morphometrics, 346 bp of sequence from the control region of mitochondrial DNA, and five nuclear microsatellite loci in various collections of *Z. hudsonius* . Morphometric analysis suggested that *Z.h. preblei*

was not a distinct subspecies, although the literature did not suggest complete distinctiveness of such traits. The limited mitochondrial DNA data set showed that all *Z.h. preblei* haplotypes were also found in *Z.h. campestris* . Microsatellite variability across the five loci suggested low level of structuring and evidence of past genetic bottlenecks. Hence, the authors suggested synonymization of *Z.h. preblei* , *Z.h. intermedius* , and *Z.h. campestris* .

My evaluation of this study is that too little genetic variation was sampled, that data analyses were tested using too few metrics against an improperly formulated set of expectations, and that the authors may have had a biased viewpoint. I was sufficiently critical of the Ramey et al. study that colleagues and I submitted a comment to the U.S. Fish and Wildlife Service on April 28, 2005; I refer you and the expert panel to that comment. When Ramey et al. subsequently published their manuscript in *Animal Conservation* , I joined colleagues and submitted a more broadly considered, critical response; I include a copy of Vignieri et al. (2006), now in press in the same journal.

Comments on King et al. Following many critical comments on the Ramey et al. study, the Service appropriately sought a more definitive study. King et al. obtained a larger sample of individuals (n = 348), surveyed a larger portion of the mitochondrial DNA molecule (1380 bp, including the control region and cytochrome *b*), and a larger collection of microsatellite markers (21 loci). They surveyed six collections of *Z.h. preblei* and seven collections representing four other subspecies. Appropriately, they tested the distribution of genetic variability against defensible criteria, i.e., significant phylogenetic separation of mitochondrial DNA haplotypes between subspecies, combined with concurrent phylogeographic structuring for nuclear DNA markers.

Variation at the large collection of microsatellite markers showed strong phylogeographic structuring of *Z. hudsonius* , which King et al. assessed with a variety of analytic tools. Perhaps of greatest interest, King et al. applied the assignment test to determine the number of clusters found in the microsatellite data and to determine the membership of individuals' multilocus genotypes within clusters. Three clusters were observed, (1) *Z.h. preblei* , (2) *Z.h. campestris* and *Z.h. intermedius* , and (3) *Z.h. pallidus* and *Z.h. luteus* . Results of the assignment test showed 100% correct assignment to the three subspecific clusters. Geographic structuring also was noted within *Z.h. preblei* and *Z.h. intermedius* . The assignment test showed 99.7% correct assignment to the seven clusters that included all subspecies and the geographic isolates for *Z.h. preblei* and *Z.h. intermedius* . D_a distances among population pairs showed the north and south isolates of *Z.h. preblei* branching distinctly from all other populations with high bootstrap support. F_{ST} values among population pairs ranged from 0.10 to 0.34, although the authors correctly noted that because F_{ST} cannot exceed 0.50, it is not a particularly useful metric with highly variable microsatellite data. Analysis of molecular variance quantified 17.4% of variation between subspecies and 82.6% within, a higher between-subspecies partitioning than is seen in many species.

Regarding variation of mitochondrial DNA, Ramey et al. did not examine a haplotype network, a surprising omission in a taxonomic study. King et al.'s haplotype network showed no shared haplotypes among subspecies at either the control region or *cytb* . F_{ST} values among

population pairs ranged from 0.22 to 0.64, although again, this is not the ideal metric for the purpose. ϕ_{ST} ranged from 0.57 to 0.97, showing more variation between than within subspecies, i.e., meeting even the unlikely criterion put forward by Ramey et al. Had Ramey et al. used this metric, which is appropriate because it accounts for mutational steps, their inferences likely would have come out differently. Population trees showed near-complete lineage sorting, although branch lengths were short.

Overall, following generation of more data and more thorough analysis than the Ramey et al. study, King et al. rejected the null hypothesis, finding that *Z.h. preblei* is genetically distinct. Synonymization of three subspecies, as proposed by Ramey et al., is not justified. I find this a well designed and executed study, and highly defensible, one that would serve well as the basis for a biological opinion under the endangered Species Act.

Comparison of Ramey et al. and King et al. studies. You and the expert panel presumably will ask why the results of the two studies differed. There are several reasons. First, while Ramey et al. sampled *individuals* as available from across the range, King et al. sampled individuals *as members of populations* across the range of the species. Second, King et al. surveyed a greater sampling of both the mitochondrial genome (346 vs. 1380 bp, from both rapidly and slowly evolving regions) and the nuclear genome (5 vs. 21 loci). King et al. used a different and more defensible set of analytic techniques: (1) haplotype networks showing relatedness of mitochondrial variants, as opposed to trees which do not, and (2) use of R_{ST} and ϕ_{ST} metrics, which reflect evolutionary relationships of microsatellite alleles, as opposed to F_{ST} which does not. Ramey et al. used an inappropriate criterion for uniqueness of subspecies, while King et al. recognized the long-standing empirical observation that variation within valid taxonomic groups may exceed that between groups. These differences in experimental design and execution all argues for the rigor of the King et al. study over that of the Ramey et al. study. Hence, I argue that the inferences of the King et al. study are defensible, while those of the Ramey et al. study are not.

Summary recommendation. I find the King et al. study of phylogeographic structuring of *Zapus hudsonius* to be well designed, executed, and reported. I agree with its inference that the Prebles meadow jumping mouse *Zapus hudsonius preblei* is a valid taxonomic unit. Hence, I urge you and the expert panel to reach a finding against the delisting of *Z.h. preblei* under the Endangered Species Act.

Once again, I am pleased to have had the opportunity to offer my comments on the proposed delisting of Preble's meadow jumping mouse. I wish the expert panel every success in reaching a scientifically justifiable finding regarding the delisting issue. You may reach me at 540-231-3257 or at ehallerm@vt.edu if you would value interactive discussion.

Sincerely,

Eric M. Hallerman

Eric M. Hallerman
Professor

Attachment: Vignieri, S.N., E.M. Hallerman, B.J. Bergstrom, D.J. Hafner, A.P. Martin, P. Devers, P. Grobler, and N. Hitt. 2006. Response to 'Mistaken view of taxonomic validity undermines conservation of an evolutionarily distinct mouse' by Ramey et al. (2005). Animal Conservation, in press. <<Vignieri et al_proof.pdf>>

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Response to 'Mistaken view of taxonomic validity undermines conservation of an evolutionarily distinct mouse' by Ramey *et al.* (2005)

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In a study self-defined in its introductory paragraph as an effort to roll back US Endangered Species Act (US-ESA) protection for a geographically isolated and currently recognized subspecies, in order to avoid misallocating financial and logistical resources, Ramey *et al.* (2005; hereafter REA) propose to synonymize the threatened Preble's meadow jumping mouse *Zapus hudsonius preblei* with two currently unlisted subspecies, the prairie jumping mouse *Zapus hudsonius intermedius* and the Bear Lodge meadow jumping mouse *Zapus hudsonius campestris*. They state a priori that their intention is to reach a conclusion that would be 'in the best interest of biodiversity conservation' and they subsequently argue that the data they present in support of their recommended synonymy is cast in a light of unbiased hypothesis testing (REA). Despite these stated claims, they dismiss the geographic isolation of this population as unimportant, ignore most of the diagnostic characters initially cited in the taxon's original description by Krutzsch (1954), conclude without data or citation a lack of ecological distinctiveness of this population, and finally misinterpret the morphological and molecular data they present.

Zapus hudsonius preblei is currently a recognized taxon and a legally protected subspecies, thus, we regard its geographic and genetic isolation, occurrence in an ecoregion distinct from that of conspecifics (Chapman *et al.*, 2004), and formally describe distinctive phenotypes of pelage and skull shape (Krutzsch, 1954) as operative hypotheses that must be explicitly disproven for synonymy to be accepted. REA proposed synonymy of *Z. h. preblei* based on four main lines of evidence – ecological differentiation, cranial morphology and analyses of mitochondrial DNA and nuclear microsatellites – and implied that their study should serve as a model of a 'conceptually sound and consistent methodological approach' for evaluating the genetic basis for listing under the US-ESA. We find that despite the potential for objective interpretation, REA reached conclusions that were neither justified by the narrow scope of their study nor supported by the data they presented. Instead, we argue that their own data support the current classification of *Z. h. preblei* as a separate evolutionary unit and a genetically distinguishable subspecies.

It is impossible to predict future patterns of speciation; thus, in our efforts to preserve biodiversity, we must seek to maximize evolutionary potential through the protection of populations on separate evolutionary trajectories (O'Brien & Mayr, 1991; Hey *et al.*, 2003). Given that the most important aspect of preserving biodiversity is protecting evolutionary potential, we are concerned that the erroneous application and interpretation of morphometric, genetic and ecological information presented by REA in an effort to subsume an evolutionarily distinctive population will not only undermine efforts to conserve this taxon but also serve as a misleading precedent applied to broader conservation programs.

Ecological analysis

REA dismiss the isolation of *Z. h. preblei* from conspecific populations, particularly *Z. h. campestris*, as merely 160 km, yet, this 160-km swath of non-habitat separating the

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northern Front Range foothills from the Black Hills foothills is the widest separation between any two subspecies of *Zapus hudsonius* (Cryan, 2004) and as such constitutes a sufficient 'primary isolating mechanism' to stop or significantly reduce gene flow, a necessary criterion for the definition of a subspecies (Whitaker, 1970; O'brien & Mayr, 1991). Additionally, it has previously been established that substantial environmental differences occur between the ranges of each of these subspecies. *Z. h. preblei* is restricted to the grama–buffalo grass association, whereas *Z. h. campestris* is found in wheatgrass–needlegrass or grama–needlegrass–wheatgrass associations (Küchler, 1970). The inarguably different environments of these disjunct populations (Chapman *et al.*, 2004; Cryan, 2004) make it likely that, in the absence of significant gene flow, ecological phenotype has diverged between them. Given this geographic and environmental separation, we argue that the potential for ecological differentiation among these populations is high.

REA ignore this most conservative expectation and assume that a lack of studies to test specifically for ecological differentiation among subspecies is equivalent to an actual lack of ecological differentiation. Further, while REA (pp. 330–331, 339–340) represent their ecological analysis as a 'method' with 'results,' they presented nothing that could be interpreted as a 'test' of 'ecological exchangeability.' REA claim to have 'examined the literature' for evidence of ecological differences between subspecies, but they neither provided detailed methods for the selection and evaluation of articles nor supported their assertion with *any* type of statistical analysis. REA admit their 'absence of evidence' is not 'evidence of absence'; their conclusion of 'ecological exchangeability' is an unsupported opinion.

A search covering 1965–2005 on the ISI Web of Knowledge (http://portal17isiknowledge.com) produced only six studies (Bain & Shenk, 2002; Schorr & Davies, 2002; Brook, Zint & De Young, 2003; Conner & Shenk, 2003; Meaney *et al.*, 2003; Ramey *et al.*, 2005), including that of REA, for '*Z. h. preblei*' or 'Preble's meadow jumping mouse,' none of which tested ecological phenotype, and no studies for '*Z. h. campestris*,' '*Z. h. intermedius*' or their respective common names. Clearly, the question of ecological exchangeability among these subspecies simply has not been posed. The lack of peer-reviewed publications on the ecology of *Z. hudsonius* subspecies (e.g. life-history characteristics, population dynamics and viability, and habitat selection; Cryan, 2004) indicates that solid research on these populations is needed before *any* conclusions can be reached about their ecological distinctiveness or exchangeability. We reject REA's claim that they conducted a test for ecological exchangeability and stress that until the question of ecological exchangeability is investigated directly, this line of inquiry is uninformative as to the question of divergence among these taxa.

Morphometric analysis

Krutzsch (1954) described 11 characters that distinguished the disjunct population of *Z. hudsonius* along the Colorado (CO) and Wyoming (WY) Front Ranges from its most similar conspecific, *Z. h. campestris* of the Black Hills–Missouri Plateau; five of these were qualitative descriptions of pelage and six were skull characteristics. The six skull characters included interorbital breadth, size and shape of auditory bullae, width and shape of incisive foramina, and degree of inflation of the frontal region. REA examined none of the pelage characters, and of the nine cranial measurements REA examined, only one – interorbital breadth – was among the six cranial characters actually cited by Krutzsch as distinguishing *Z. h. preblei* from *Z. h. campestris*. Of the cranial metrics REA used, five included greatest length of skull (GLS) or measures highly correlated with GLS, and the other four were measures of skull breadth. Interestingly, of the 36 pairwise Pearson correlation coefficients among these nine variables, 26 were significant at $P<0.001$ (two-tailed α; Minitab, 1996; raw data from U.S. Fish and Wildlife Service (USFWS, 2004).

No univariate or multivariate analysis of these metrics could possibly have resolved the incisive foramina, auditory bullae or frontal inflation size/shape characters cited by Krutzsch (1954) as constituting 'considerable differences.' Therefore, REA have conducted an incomplete test of the morphologic hypothesis put forth by Krutzsch. Importantly, the sole univariate character cited by Krutzsch that REA did examine, interorbital breadth, was found to be narrower in *Z. h. preblei* than in *Z. h. campestris*, as described in the definitive findings (Krutzsch, 1954). Thus, the small fraction of Krutzsch's morpho-taxonomic hypothesis actually tested by REA confirmed Krutzsch's initial findings of distinctiveness for *Z. h. preblei*. Oddly, their conclusions imply the opposite. REA apparently viewed a multivariate statistical test of a standard set of morphologic variables, although incomplete and intercorrelated, as a substitute for attempting to quantify the specific shape differences noted by a trained morpho-taxonomist. One should not expect such an arbitrary, hypothesis-free approach to resolve subspecies relationships (Gift & Stevens, 1997; Poe & Wiens, 2000); examples of the failure of this blind approach abound, even when comparing full species (e.g. Poole, Carpenter & Simms, 1980; Zink 1988; e.g. Barratt *et al.*, 1997).

Molecular genetic analyses

Mitochondrial DNA

Although mtDNA is still occasionally used as the sole locus in phylogenetic studies, it is accepted that if doing so, sequence length should be maximized as any single locus will be subject to variation of d, the number of substitutions per site, and this variation will be reduced as the number of sites sequenced per gene is increased (Arbogast *et al.*, 2002). A much more accepted and accurate approach for obtaining a gene genealogy (gene tree) reflective of the true lineage genealogy ('species' tree), however, is the inclusion of multiple independent loci (Edwards & Beerli, 2000). The examination of divergence patterns across multiple loci decreases

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the coalescent variation (the stochastic variance in gene divergence times which arises due to genetic drift, Arbogast *et al.*, 2002) and thus vastly improves the estimate of the true history of a lineage. When only a single locus is used to construct a phylogeny, discordances between this single locus gene tree and the actual species tree will be expected due to ancestral polymorphism and incomplete lineage sorting (Maddison, 1997; Arbogast *et al.*, 2002). These processes are expected to be even more pronounced in recently diverged lineages and those with structured populations (Wakeley, 2000, 2001), as would be expected in this habitat-specific subspecies group. Despite these well-understood expectations, REA used only a single, short [346 base pairs (bp)]) region of the mtDNA control region to test for divergence among the *Z. hudsonius* subspecies group and then treated the patterns of divergence observed within this single region as equivalent to the patterns of divergence among the subspecies.

We caution that the mtDNA data presented by REA should be viewed as preliminary. However, we find that in their current state they are nonetheless consistent with the expectation of incomplete lineage sorting and are indicative of divergence among the subspecies examined. Although bootstrap support for the split between the *Zapus hudsonius luteus*/*Zapus hudsonius pallidus* and *Z. h. preblei*/*Z. h. campestris*/*Z. h. intermedius* clades was high, support was quite low for REA's terminal clades ($\leq$ 50–68%); thus terminal branching patterns within this phylogeny should be considered hypotheses with little support (we note in particular that terminal branch support for clades that grouped *Z. h. preblei* with *Z. h. campestris* appeared to receive support of <52%). Nonetheless, all individuals identified a priori as *Z. h. preblei* grouped within a single clade. REA put forth reciprocal monophyly (Moritz, 1994*b*) as the sole criteria for accepting divergence among subspecies; however, given the expectation of incomplete lineage sorting, this requirement was overly stringent, and it being the sole criteria for acceptance of divergence increased the likelihood that REA would conclude that no differences exist among subspecies. Notably, and consistent with an understanding that incomplete lineage sorting can complicate the understanding of phylogenetic history, Moritz (1994*a*) modified his proposal of reciprocal monophyly with the suggestion that significant, but not necessarily absolute, separation of alleles among populations is an appropriate indicator of the presence of distinct, taxonomically recognizable entities.

Although we find the current phylogeny generated by REA to be preliminary, the marked differences in haplotype frequencies observed among the five subspecies clearly support divergence. In order to further explore the pattern of haplotype frequencies among the different subspecies, we designated each observed haplotype (from REA) to the subspecies within which it occurred with the highest frequency (calculated from Appendix 2 of REA); for example, all L and L/PAL haplotypes were assigned as '*luteus* haplotypes' (with frequencies in *Z. h. luteus* of 1.00), although they also occur in *pallidus* and *campestris* at much lower frequencies (0.059 and 0.129, respectively; Table 1). 'Contaminant' haplotypes may result from incomplete lineage sorting, migration from adjacent subspecies or misidentification of individuals at subspecific boundaries. Although both incomplete lineage sorting and migration of individuals from adjacent subspecies would be expected, other cases of supposed 'contamination' more likely result from misidentification of individuals. For example (Appendix 2 of REA), three individuals of '*Z. h. intermedius*' from Harding Co. in north-western South Dakota (Fig. 1) with the C5/INT13 haplotype (designated as a '*campestris* haplotype') are mapped by REA (their fig. 4) as occurring within the range of *Z. h. campestris*, and two individuals of '*Z. h. pallidus*' from Clay Co. in extreme south-eastern South Dakota (Fig. 1) with the PAL1/INT15 haplotype (designated as an '*intermedius* haplotype') are the only '*Z. h. pallidus*' found within the range of *Z. h. intermedius*, north of the Missouri River. Even if we assume these individuals were correctly assigned to subspecies, *Z. h. preblei*, *Z. h. luteus*, *Z. h. intermedius* and *Z. h. pallidus* exhibited low frequencies of 'contaminant' haplotypes of all types, whereas *Z. h. campestris* contained an admixture of haplotypes (Table 1, Fig. 1).

Table 1 Frequency of subspecies characteristic haplotypes (assigned to subspecies based on highest frequency of occurrence) within five subspecies of *Zapus hudsonius*

	Subspecies				
Haplotype	*Preblei*	*Luteus*	*Intermedius*	*Pallidus*	*Campestris*
Preblei	**1.000**				[0.226]
Luteus		**1.000**		[0.059]	[0.129]
Intermedius			**0.915**	(0.059)	(0.258)
Pallidus			[0.021]	**0.882**	
Campestris			(0.064)		**0.387**

The frequency at which each subspecific haplotype is found within each subspecies is shown in boldface along the diagonal, squares indicate ancestral haplotypes shared likely due to incomplete lineage sorting, ovals indicate results of possible migration or mistaken subspecific identification (based on geographic location)

The unique admixture of haplotypes in *Z. h. campestris* may indicate a previously more widespread distribution (allowing retention of ancestral haplotypes), may simply reflect that subspecies' geographic position adjacent to three other subspecies (opportunities for migration and misidentification), or a combination of both factors. Notably, no contaminant haplotypes were found in *Z. h. preblei*, and although '*preblei* haplotypes' were also found in the highly admixed *Z. h. campestris*, the haplotype frequency differences between these subspecies were striking (Fig. 1, Table 1 and REA fig. 3). This pattern of significant haplotype frequency differences occurring in conjunction with a lack of reciprocal monophyly for two closely related lineages is consistent with the process of incomplete lineage sorting wherein ancestral polymorphism of haplotypes is retained across divergent lineages at low frequencies (Avise, 2000). Such incomplete sorting of haplotypes is not only expected theoretically, but has also been well documented in a wide

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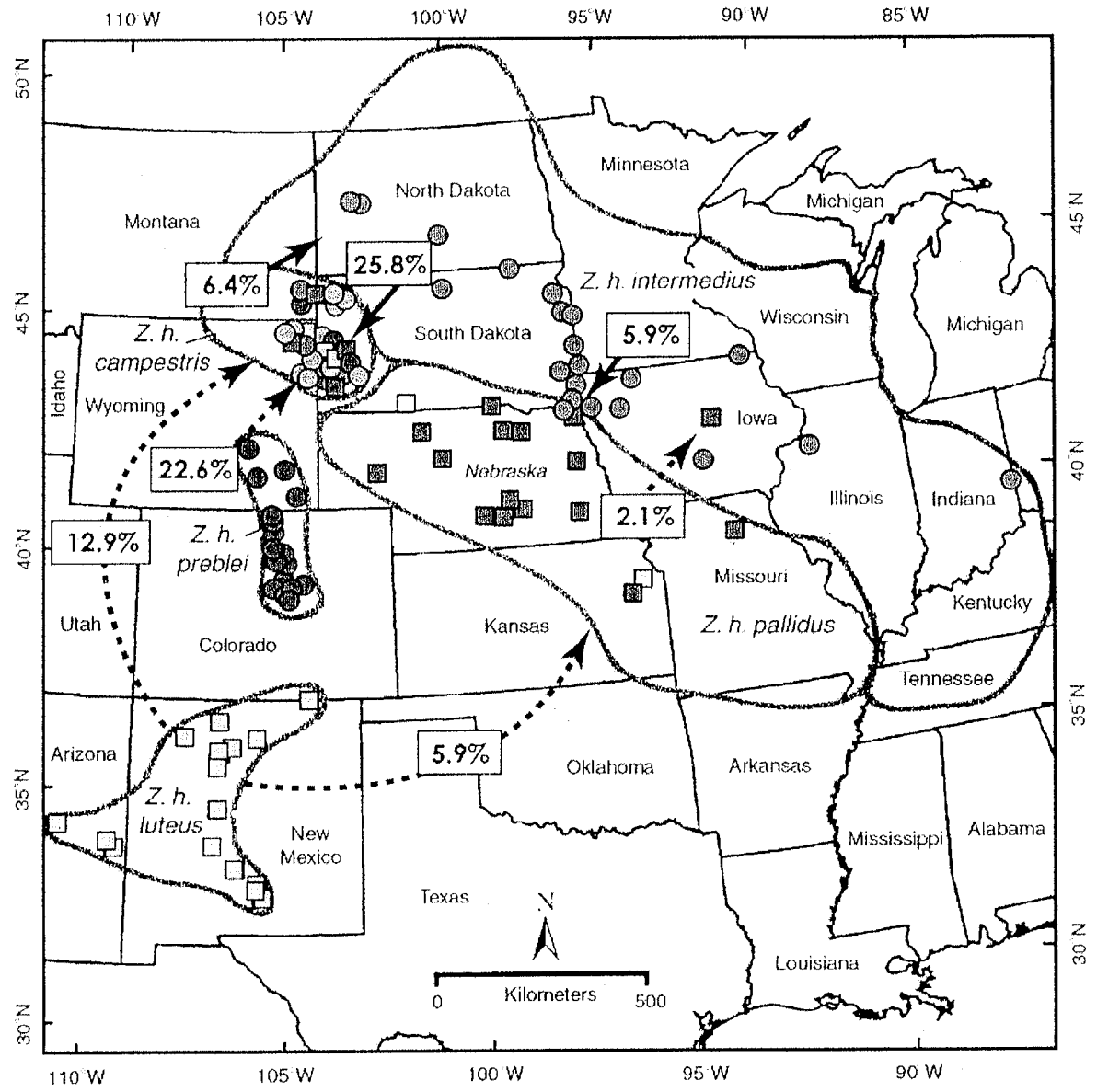

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Figure 1 Distribution of mtDNA haplotypes among five subspecies of *Zapus hudsonius*. Squares = *pallidus-luteus* lineage, circles = *intermedius-campestris-preblei* lineage (from fig. 3 of Ramey *et al.* 2005). Colors (modified from the original figure) indicate haplotype assignment to subspecies (see text and Table 1). Percentages of haplotypes characteristic of one subspecies and found in another are indicated within boxes next to arrows. Solid arrows indicate probable migration or mistaken subspecific identification of samples; dotted arrrows indicate probable shared-ancestral haplotypes due to incomplete lineage sorting.

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variety of organisms, including taxa that are clearly separate biological species (Avise, 2000).

Given the availability of rapid DNA sequencing technology, universal primers for mtDNA amplification and numerous nuclear loci for mammals (48 reported by Yang & Nielsen, 1998), the short sequence of the single mtDNA locus used by REA represents a minimal effort toward revealing patterns of divergence in this group and should be observed as only a preliminary foray into its true evolutionary history. Many studies investigating similar questions of lineage divergence have used much higher standards and these should be viewed as more solid models for taxonomic investigation. For example, Roca *et al.* (2001) used 1732 bp from four nuclear DNA genes to separate African forest elephants from savannah elephants as separate species. Culver *et al.* (2000) used 891 bp of mitochondrial DNA and 10 DNA microsatellites to collapse 15 historically recognized subspecies of puma into six subspecies, and J. W. Jones *et al.* (unpubl. data) used 1900 bp of combined mitochondrial and nuclear DNA sequences and 10 DNA Q3

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microsatellites to distinguish populations of endangered freshwater mussels as either species or subspecies. These studies also used geography, life history, behavior and morphology to corroborate their findings. Given the strength of the arguments for the use of multiple loci in phylogenetic studies and the prevalence of numerous studies demonstrating much higher standards of data inclusion, the single-locus, short sequence approach used by REA should be viewed as precursory and most certainly should not be presented as an adequate basis for the making of taxonomic decisions regarding a listed taxon.

The taxa investigated by REA clearly violate an assumption of the MDIV test for gene flow among subspecies, the assumption of equal effective population size (n_e). Nevertheless, if we assume their estimates are generally accurate, the degree of gene flow between *Z. h. preblei* and *Z. h. campestris* is very low, an unscaled rate of 0.000033 to 0.0000032 individuals per generation. This rate does not qualify as homogenizing gene flow. Natural hybridization among well-differentiated species can occur at rates higher than this (e.g. Campton & Utter, 1985; Arnold, 1992; Roques, Sevigny & Bernatchez, 2001), and low levels of gene flow do not preclude local adaptation (Broggi *et al.*, 2005). Although complete introgressive hybridization (i.e. hybrid swarms) may exclude hybridized populations from the units considered for listing under the US-ESA (Allendorf *et al.*, 2004), REA quite clearly demonstrate that this level of introgression is *not* occurring among *Z. h. preblei* and other subspecies.

Microsatellites

Similar to the analysis of mtDNA sequence data, REA used too few loci in the microsatellite analysis to ensure high resolution. Smouse & Chevillon (1998) state that 'large numbers of polymorphic loci' are required 'to assign individuals to their correct population' and emphasize that there is a positive relationship between the number of populations in question and the number of loci required to place individuals correctly. In initially describing the STRUCTURE method used by REA, Pritchard, Stephens & Donnelly (2000) were unable to acquire a clear estimate for *K* (the number of populations represented within the sample) with their simulated dataset using five polymorphic loci. Further, they concluded that 'the accuracy of assignment depends on ... the number of loci [which will affect the accuracy of q_{MAX} (likelihood of assignment of an individual to a given cluster)].' Although locus availability is often a problem, as of 2003 there were at least eight additional microsatellite loci for *Zapus* spp. (Vignieri, 2003) available for use by REA.

Given the expected low resolving power of the microsatellite data, REA's results are surprisingly strong in support of differentiation of *Z. h. preblei* from the other subspecies. F_{ST} values that are significantly different from zero indicate that gene flow among the compared populations is limited enough to result in genetic divergence. F_{ST} values observed among *Z. hudsonius* subspecies were significant for all pairwise comparisons, indicating that variation in allele frequencies among subspecies was greater than that within subspecies (Wright, 1951; Weir & Cockerham, 1984), thus the subspecies are genetically diverged. Although REA argue that their observed F_{ST} values are low (0.07–0.16), they are well within the range generally observed among subspecies in mammals (gray wolf 0.168, Roy *et al.*, 1994; African buffalo 0.059, Van Hooft, Groen & Prins, 2000, jaguar 0.065, Eizirik *et al.*, 2001). Further, REA report high per-locus polymorphism and high values of within-population heterozygosity, H_s (0.69–0.94). Considering the value of F_{ST} can be no larger than $1 - H_s$ (Hedrick, 1999), even with complete differentiation, the highest absolute F_{ST} we would expect for the loci used by REA ranges from 0.06 to 0.31, and thus the F_{ST} values observed among subspecies are relatively high.

Strong support for differentiation among subspecies is also found in the STRUCTURE analysis. Although resolving power with five loci is limited, q_{MAX} for both *Z. h. preblei* populations was quite high ($q_{MAX} = 0.85$ for the northern population and 0.86 for the southern population). All other subspecies had lower q_{MAX} values, including *Z. h. luteus* (0.67), whose distinctiveness REA do not question. Similarly, correct assignment proportions for both northern (42.9%) and southern (54.5%) populations of *Z. h. preblei* were considerably higher than those observed in any other subspecies, including *Z. h. luteus* (only 21.9% of individuals correctly assigned). Additionally, 95% of the northern population and 94% of the southern population of *Z. h. preblei* were assigned to two clusters (2 and 5) that had very few individuals assigned from any of the other subspecies (REA table 6). Given the low resolving power of the loci used by REA, the relatively high proportion of correct assignment observed in *Z. h. preblei* populations provides further strong evidence of differentiation.

Use and Interpretation of AMOVA

REA use AMOVA as a measure of distinctiveness of *Z. h. preblei*, and set the criterion that there must 'be greater molecular variance among than within subspecies.' Results from mtDNA sequences showed that 18.5–37% of variation was found between subspecies, and microsatellite data indicate that 7.5–9% of variation occurred between populations. Although the authors do not present a significance value for the AMOVA test, they claim that *Z. h. preblei* fails these tests of genetic uniqueness. However, the within-population component of total genetic diversity may exceed the between-population component even when comparing separate species. For example, Leibers, Helbig & De Knijff (2001; using mtDNA sequence data) found that only 26.8% of the total diversity among gull populations resides among acknowledged species. Using microsatellite data, Grobler *et al.* (2005) found that only 29.2% of the total variation among blue and black wildebeest populations occurs between species. Thus, it is not necessarily expected that an AMOVA-based analysis of subspecies, or even species, will reveal more diversity among than within subspecies. The

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criterion used by REA was dubious at best, and the conclusion drawn from failure to meet this criterion is not valid.

Conclusion

The definition of taxonomic groups has long been an area of contention. Species concepts are abundant and continuously debated (a recent count listed 24; Mayden, 1997), and concepts of subspecies are even less well defined. Given the uncertainty present in both the definition of taxonomic status and the identification of such, in our efforts to preserve biodiversity we should be striving to protect populations of organisms that are on separate evolutionary trajectories rather than debating taxonomic definitions (Waples, 1991; Hey *et al.*, 2003). This desire has been expressed in both the literature and the intent of government policy where evolutionarily significant units (ESUs) and distinctive population segments (DPSs) have been identified as groups worthy of protection. Within the US-ESA, species are defined as 'any subspecies of fish or wildlife or plants, and any *distinct population segment* of any species of vertebrate fish or wildlife which interbreeds when mature' [16 U.S.C., Sec. 1532(16)] and it states that the definition of such groups should be determined based 'solely on the best available science.' Clearly, the intent of conservation policy is to protect populations identified, in a scientifically rigorous way, as evolutionarily distinctive. Given the clarity of this intention, we find REA's recommendation of synonymy of *Z. h. preblei* curious and unjustified.

We firmly believe that no single approach should be used as a 'taxonomic litmus test' for taxa of conservation concern. However, for cases where such testing is appropriate, we offer a simple alternative hypothesis-testing approach based on the understanding that conservation of biodiversity requires conservation of groups that are evolutionarily distinct. Given this goal, we can address questions of conservation units based on this null hypothesis: These populations of individuals represent a readily interbreeding, undifferentiated unit with shared adaptations and a common evolutionary trajectory. What we are truly interested in revealing is whether there is *any* evidence that a given group is evolutionarily unique and therefore an important component of global biodiversity. Considering the data on *Z. hudsonius* subspecies presented by REA and other published information on the taxa and their environments we have discussed, we find the null hypothesis, that this group represents one readily interbreeding, undifferentiated unit, can be rejected, and the alternate hypothesis, that the populations currently classified as subspecies represent unique evolutionary entities, can be accepted across *all* of REA's informative lines of evidence. Gene flow between these disjunct subspecies is exceedingly low; there is evidence that *Z. h. preblei* is diverged in morphology and strong evidence that it is substantially diverged in mtDNA haplotype frequencies and microsatellite allele frequencies and allelic distribution.

Because REA assert a challenge to Preble's meadow jumping mouse's current classification as a subspecies, the burden of proof is upon them to provide clear, solid evidence that this taxon is not evolutionarily distinct and thus its subspecific classification is unwarranted. Contrary to REA's stated conclusions, we find no evidence supporting their extreme recommendation of synonymy and instead conclude that their evidence offers further support for the classification of *Z. h. preblei* as a unique subspecies and a distinct evolutionary unit worthy of the protection it is currently afforded. Finally, we caution that vague questions of 'taxonomic validity' can undermine the intent to protect evolutionarily distinct units and we urge that this study not be considered a precedent for evaluation of validity in taxa of conservation concern.

References

Allendorf, F.W., Leary, R.F. & Hitt, N.P., *et al.* (2004). Intercrosses and the US Endangered Species Act: should Q4 hybridized populations be included as westslope cutthroat trout? *Conserv. Biol.* **18**, 1203–1213.

Arbogast, B.S., Edwards, S.V., Wakeley, J., Beerli, P. & Slowinski, J.B. (2002). Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annu. Rev. Ecol. Syst.* **33**, 707–740.

Arnold, M.L. (1992). Natural hybridization as an evolutionary process. *Annu. Rev. Ecol. Syst.* **23**, 237–261.

Avise, J.C. (2000). *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.

Bain, M.R. & Shenk, T.M. (2002). Nests of Preble's meadow jumping mouse (*Zapus hudsonius preblei*) in Douglas County, Colorado. *Southwest. Nat.* **47**, 630–633.

Barratt, E.M., Deaville, R. & Burland, T.M., *et al.* (1997). DNA answers the call of pipistrelle bat species. *Nature* **387**, Q5 138–139.

Broggi, J., Hohtola, E., Orell, M. & Nilsson, J.A. (2005). Local adaptation to winter conditions in a passerine spreading north: a common-garden approach. *Evolution* **59**, 1600–1603.

Brook, A., Zint, M. & De Young, R. (2003). Landowners' responses to an Endangered Species Act listing and implications for encouraging conservation. *Conserv. Biol.* **17**, 1638–1649.

Campton, D.E. & Utter, F.M. (1985). Natural hybridization between steelhead trout (*Salmo-gairdneri*) and coastal cutthroat trout (*Salmo-clarki-clarki*) in 2 Puget sound streams. *Can. J. Fish. Aqua. Sci.* **42**, 110–119.

Chapman, S.S., Bryce, S.A. & Omernik, J.M., *et al.* (2004). *Ecoregions of Wyoming, color poster with map, descriptive text, summary tables, and photographs*. Reston, VA: United Q6 States Geological Survey.

Conner, M.M. & Shenk, T.M. (2003). Distinguishing *Zapus hudsonius preblei* from *Zapus princeps princeps* by using repeated cranial measurements. *J. Mammal.* **84**, 1456–1463.

Cryan, P.M. (2004). *Synthesis of existing information on meadow jumping mice (Zapus hudsonius) in the northern*

(bwuk ACV 38 1..8.PDF 4/24/2006 9:12:34 AM 497096 Bytes 8 PAGES Operator=)
ACV 38

great plains. An informative review compiled for the U.S Fish and Wildlife Service, Region 6, Denver, CO: 1–27.

Culver, M., Johnson, W.E., Pecon-Slattery, J. & O'Brien, S.J. (2000). Genomic ancestry of the American puma (*Puma concolor*). *J. Hered.* **91**, 186–197.

Edwards, S.V. & Beerli, P. (2000). Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* **54**, 1839–1854.

Eizirik, E., Kim, J.H. & Menotti-Raymond, M., *et al.* (2001). Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). *Mol. Ecol.* **10**, 65–79.

Gift, N. & Stevens, P.F. (1997). Vagaries in the delimitation of character states in quantitative variation – an experimental study. *Syst. Biol.* **46**, 112–125.

Grobler, J.P., Hartl, G.B. & Grobler, N., *et al.* (2005). The genetic status of an isolated black wildebeest (*Connochaetes gnou*) population from the Abe Bailey Nature Reserve, South Africa: microsatellite data on a putative past hybridization with blue wildebeest (*C. taurinus*). *Mammal. Biol.* **70**, 35–45.

Hedrick, P.W. (1999). Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution* **53**, 313–318.

Hey, J., Waples, R.S., Arnold, M.L., Butlin, R.K. & Harrison, R.G. (2003). Understanding and confronting species uncertainty in biology and conservation. *Trends Ecol. Evol.* **18**, 597.

Krutzsch, P.H. (1954). North American jumping mice (genus *Zapus*). *Univ. Kansas Publ. Mus. Nat. Hist.* **7**, 349–472.

Küchler, A.W. (1970). Potential natural vegetation (revised 1985). In *National atlas of the United States of America*: 89–92. United States Department of the Interior, United States Geological Survey, 38077-AH-NA-07M-00:1–417.

Leibers, D., Helbig, A.J. & De Knijff, P. (2001). Genetic differentiation and phylogeography of gulls in the *Larus cachinnans-fuscus* groups (Aves: Charadriiformes). *Mol. Ecol.* **10**, 2447–2462.

Maddison, W.P. (1997). Gene trees in species trees. *Syst. Biol.* **46**, 523–536.

Mayden, R.L. (1997). A hierarchy of species concepts: the denouement in the saga of the species problem. In *Species: the units of biodiversity*: 381–424. Claridge, M.F. (Ed.). London: Chapman & Hall.

Meaney, C.A., Ruggles, A.K., Lubow, B.C. & Clippinger, N.W. (2003). Abundance, survival, and hibernation of Preble's meadow jumping mice (*Zapus hudsonius preblei*) in Boulder County, Colorado. *Southwest. Nat.* **48**, 610–623.

Minitab, I. (1996). *Minitab reference manual*. State College, PA: Minitab, Inc.

Moritz, C. (1994*a*). Applications of mitochondrial-DNA analysis in conservation – a critical review. *Mol. Ecol.* **3**, 401–411.

Moritz, C. (1994*b*). Defining evolutionarily-significant units for conservation. *Trends Ecol. Evol.* **9**, 373–375.

O'Brien, S.J. & Mayr, E. (1991). Bureaucratic mischief – recognizing endangered species and subspecies. *Science* **251**, 1187–1188.

Poe, S. & Wiens, J.J. (2000). Character selection and the methodology of morphological phylogenetics. In *Phylogenetic analysis of morphological data*: 20–36. Wiens, J.J. (Ed.). Washington, DC: Smithsonian Institution Press.

Poole, W.E., Carpenter, S.M. & Simms, N.G. (1980). Multivariate analyses of skull morphometrics from the two species of grey kangaroos, *Macropus giganteus* shaw and *M. fuliginosus* (Desmarest). *Aust. J. Zool.* **69**, 591–605.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Ramey, R.R. II, Liu, H.P., Epps, C.W., Carpenter, L.M. & Wehausen, J.D. (2005). Genetic relatedness of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA and microsatellite DNA: implications for taxonomy and conservation. *Anim. Conserv.* **8**, 329–346.

Roca, A.L., Georgiadis, N., Pecon-Slattery, J. & O'Brien, S.J. (2001). Genetic evidence for two species of elephant in Africa. *Science* **293**, 1473–1477.

Roques, S., Sevigny, J.M. & Bernatchez, L. (2001). Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the north-west Atlantic: a rare marine example. *Mol. Ecol.* **10**, 149–165.

Roy, M.S., Geffen, E., Smith, D., Ostrander, E.A. & Wayne, R.K. (1994). Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. *Mol. Biol. Evol.* **11**, 553–570.

Schorr, R.A. & Davies, R. (2002). Grey flesh fly (*Wohlfahrtia vigil*) parasitism of a Preble's meadow jumping mouse (*Zapus hudsonius preblei*). *J. Wildl. Diseases* **38**, 604–606.

Smouse, P.E. & Chevillon, C. (1998). Analytical aspects of population-specific DNA fingerprinting for individuals. *J. Hered.* **89**, 143–150.

USFWS (2004). *Preble's meadow jumping mouse recovery plans*. USFWS Mountain-Prarie Region Endangered Species Program.

Van Hooft, W.F., Groen, A.F. & Prins, H.H.T. (2000). Microsatellite analysis of genetic diversity in African buffalo (*Syncerus caffer*) populations throughout Africa. *Mol. Ecol.* **9**, 2017–2025.

Vignieri, S.N. (2003). Isolation and characterization of eight highly variable microsatellite markers in the Pacific jumping mouse (*Zapus trinotatus*). *Mol. Ecol. Notes* **3**, 638–640.

Wakeley, J. (2000). The effects of subdivision on the genetic divergence of populations and species. *Evolution* **54**, 1092–1101.

Wakeley, J. (2001). The coalescent in an island model of population subdivision with variation among demes. *Theor. Popul. Biol.* **59**, 133–144.

(bwuk ACV 38 1..8.PDF 4/24/2006 9:12:34 AM 497096 Bytes 8 PAGES Operator=)
ACV 38

Waples, R.S. (1991). Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. *Mar. Fish. Rev.* **53**, 11–22.

Weir, B.S. & Cockerham, C.C. (1984). Estimating F-statistics for the analysis of population–structure. *Evolution* **38**, 1358–1370.

Whitaker, J.O. (1970). The biological subspecies: an adjunct of the biological species. *Biologist* **52**, 12–15.

Wright, S. (1951). The genetical structure of populations. *Ann. Eugenics* **15**, 323–354.

Yang, Z.H. & Nielsen, R. (1998). Synonymous and nonsynonymous rate variation in nuclear genes of mammals. *J. Mol. Evol.* **46**, 409–418.

Zink, R.M. (1988). Evolution of brown towhees: allozymes, morphometrics and species limits. *Condor* **90**, 72–82.

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Hello,
We would like to submit this manuscript, published recently in Animal Conservation, as a public comment on the proposed delisting of the Preble's meadow jumping mouse.

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Mistaken view of taxonomic validity undermines conservation of an evolutionarily distinct mouse: a response to Ramey *et al.* (2005)

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In a study self-defined in its introductory paragraph as an effort to roll back US Endangered Species Act (US-ESA) protection for a geographically isolated and currently recognized subspecies, in order to avoid misallocating financial and logistical resources, Ramey *et al.* (2005; hereafter REA) proposed to synonymize the threatened Preble's meadow jumping mouse *Zapus hudsonius preblei* with two currently unlisted subspecies, the prairie jumping mouse *Zapus hudsonius intermedius* and the Bear Lodge meadow jumping mouse *Zapus hudsonius campestris*. They stated a priori that their intention was to reach a conclusion that would be 'in the best interest of biodiversity conservation' and they subsequently argued that the data they presented in support of their recommended synonymy were cast in a light of unbiased hypothesis testing (REA). Despite these stated claims, they dismissed the geographic isolation of this population as unimportant, ignored most of the diagnostic characters initially cited in the taxon's original description by Krutzsch (1954), concluded without data or citation a lack of ecological distinctiveness of this population, and finally misinterpreted the morphological and molecular data they presented.

Zapus hudsonius preblei is currently a recognized taxon and a legally protected subspecies; thus, we regard its geographic and genetic isolation, occurrence in an ecoregion distinct from that of conspecifics (Chapman *et al.*, 2004), and formally described distinctive phenotypes of pelage and skull shape (Krutzsch, 1954) as operative hypotheses that must be explicitly disproven for synonymy to be accepted. REA proposed synonymy of *Z. h. preblei* based on four main lines of evidence – ecological differentiation, cranial morphology and analyses of mitochondrial DNA and nuclear microsatellites – and implied that their study should serve as a model of a 'conceptually sound and consistent methodological approach' for evaluating the genetic basis for listing under the US-ESA. We find that despite the potential for objective interpretation, REA reached conclusions that were neither justified by the narrow scope of their study nor supported by the data they presented. Instead, we argue that their own data support the current classification of *Z. h. preblei* as a separate evolutionary unit and a genetically distinguishable subspecies.

It is impossible to predict future patterns of speciation; thus, in our efforts to preserve biodiversity, we must seek to maximize evolutionary potential through the protection of populations on separate evolutionary trajectories (O'Brien & Mayr, 1991; Hey *et al.*, 2003). Given that the most important aspect of preserving biodiversity is protecting evolutionary potential, we are concerned that the erroneous application and interpretation of morphometric, genetic and ecological information presented by REA in an effort to subsume an evolutionarily distinctive population will not only undermine efforts to conserve this taxon but also serve as a misleading precedent applied to broader conservation programs.

Ecological analysis

REA dismissed the isolation of *Z. h. preblei* from conspecific populations, particularly *Z. h. campestris*, as merely 160 km; yet, this 160-km swath of non-habitat separating the

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northern Front Range foothills from the Black Hills foothills is the widest separation between any two subspecies of *Zapus hudsonius* (Cryan, 2004) and as such constitutes a sufficient 'primary isolating mechanism' to stop or significantly reduce gene flow, a necessary criterion for the definition of a subspecies (Whitaker, 1970; O'brien & Mayr, 1991). Additionally, it has previously been established that substantial environmental differences occur between the ranges of each of these subspecies: *Z. h. preblei* is restricted to the grama–buffalo grass association, whereas *Z. h. campestris* is found in wheatgrass–needlegrass or grama–needlegrass–wheatgrass associations (Küchler, 1970). The inarguably different environments of these disjunct populations (Chapman *et al.*, 2004; Cryan, 2004) make it likely that, in the absence of significant gene flow, ecological phenotype has diverged between them. Given this geographic and environmental separation, we argue that the potential for ecological differentiation among these populations is high.

REA ignored this most conservative expectation and assumed that a lack of studies to test specifically for ecological differentiation among subspecies is equivalent to an actual lack of ecological differentiation. Further, while REA (pp. 330–331, 339–340) represented their ecological analysis as a 'method' with 'results,' they presented nothing that could be interpreted as a 'test' of 'ecological exchangeability.' REA claimed to have 'examined the literature' for evidence of ecological differences between subspecies, but they neither provided detailed methods for the selection and evaluation of articles nor supported their assertion with *any* type of statistical analysis. REA admit their 'absence of evidence' is not 'evidence of absence', their conclusion of 'ecological exchangeability' is an unsupported opinion.

A search covering 1965–2005 on the ISI Web of Knowledge (http://portal17isiknowledge.com) produced only six studies (Bain & Shenk, 2002; Schorr & Davies, 2002; Brook, Zint & De Young, 2003; Conner & Shenk, 2003; Meaney *et al.*, 2003; Ramey *et al.*, 2005), including REA, for '*Zapus hudsonius preblei*' or 'Preble's meadow jumping mouse,' none of which tested ecological phenotype, and no studies for '*Zapus hudsonius campestris*,' '*Zapus hudsonius intermedius*' or their respective common names. Clearly, the question of ecological exchangeability among these subspecies simply has not been posed. The lack of peer-reviewed publications on the ecology of *Z. hudsonius* subspecies (e.g. life-history characteristics, population dynamics and viability, and habitat selection; Cryan, 2004) indicates that solid research on these populations is needed before *any* conclusions can be reached about their ecological distinctiveness or exchangeability. We reject REA's claim that they conducted a test for ecological exchangeability and stress that until the question of ecological exchangeability is investigated directly, this line of inquiry is uninformative as to the question of divergence among these taxa.

Morphometric analysis

Krutzsch (1954) described 11 characters that distinguished the disjunct population of *Z. hudsonius* along the Colorado (CO) and Wyoming (WY) Front Ranges from its most similar conspecific, *Z. h. campestris* of the Black Hills–Missouri Plateau; five of these were qualitative descriptions of pelage and six were skull characteristics. The six skull characters included interorbital breadth, size and shape of auditory bullae, width and shape of incisive foramina, and degree of inflation of the frontal region. REA examined none of the pelage characters, and of the nine cranial measurements REA examined, only one – interorbital breadth – was among the six cranial characters actually cited by Krutzsch as distinguishing *Z. h. preblei* from *Z. h. campestris*. Of the cranial metrics REA used, five included greatest length of skull (GLS) or measures highly correlated with GLS, and the other four were measures of skull breadth. Interestingly, of the 36 pairwise Pearson correlation coefficients among these nine variables, 26 were significant at $P<0.001$ (two-tailed α; Minitab, 1996; raw data from U.S. Fish and Wildlife Service (USFWS, 2004).

No univariate or multivariate analysis of these metrics could possibly have resolved the incisive foramina, auditory bullae or frontal inflation size/shape characters cited by Krutzsch (1954) as constituting 'considerable differences.' Therefore, REA have conducted an incomplete test of the morphologic hypothesis put forth by Krutzsch. Importantly, the sole univariate character cited by Krutzsch that REA did examine, interorbital breadth, was found to be narrower in *Z. h. preblei* than in *Z. h. campestris*, as described in the definitive findings (Krutzsch, 1954). Thus, the small fraction of Krutzsch's morpho-taxonomic hypothesis actually tested by REA confirmed Krutzsch's initial findings of distinctiveness for *Z. h. preblei*. Oddly, their conclusions imply the opposite. REA apparently viewed a multivariate statistical test of a standard set of morphologic variables, although incomplete and intercorrelated, as a substitute for attempting to quantify the specific shape differences noted by a trained morpho-taxonomist. One should not expect such an arbitrary, hypothesis-free approach to resolve subspecies relationships (Gift & Stevens, 1997; Poe & Wiens, 2000); examples of the failure of this blind approach abound, even when comparing full species (e.g. Poole, Carpenter & Simms, 1980; Zink 1988; e.g. Barratt *et al.*, 1997).

Molecular genetic analyses

Mitochondrial DNA

Although mtDNA is still occasionally used as the sole locus in phylogenetic studies, it is accepted that if doing so, sequence length should be maximized as any single locus will be subject to variation of d, the number of substitutions per site, and this variation will be reduced as the number of sites sequenced per gene is increased (Arbogast *et al.*, 2002). A much more accepted and accurate approach for obtaining a gene genealogy (gene tree) reflective of the true lineage genealogy ('species' tree), however, is the inclusion of multiple independent loci (Edwards & Beerli, 2000). The examination of divergence patterns across multiple loci decreases

the coalescent variation (the stochastic variance in gene divergence times which arises due to genetic drift; Arbogast *et al.*, 2002) and thus vastly improves the estimate of the true history of a lineage. When only a single locus is used to construct a phylogeny, discordances between this single locus gene tree and the actual species tree will be expected due to ancestral polymorphism and incomplete lineage sorting (Maddison, 1997; Arbogast *et al.*, 2002). These processes are expected to be even more pronounced in recently diverged lineages and those with structured populations (Wakeley, 2000, 2001), as would be expected in this habitat-specific subspecies group. Despite these well-understood expectations, REA used only a single, short [346 base pairs (bp)] region of the mtDNA control region to test for divergence among the *Z. hudsonius* subspecies group and then treated the patterns of divergence observed within this single region as equivalent to the patterns of divergence among the subspecies.

We caution that the mtDNA data presented by REA should be viewed as preliminary. However, we find that in their current state they are nonetheless consistent with the expectation of incomplete lineage sorting and are indicative of divergence among the subspecies examined. Although bootstrap support for the split between the *Zapus hudsonius luteus/Zapus hudsonius pallidus* and *Z. h. preblei/Z. h. campestris/Z. h. intermedius* clades was high, support was quite low for REA's terminal clades ($\leq$ 50–68%); thus terminal branching patterns within this phylogeny should be considered hypotheses with little support (we note in particular that terminal branch support for clades that grouped *Z. h. preblei* with *Z .h. campestris* appeared to receive support of <52%). Nonetheless, all individuals identified a priori as *Z. h. preblei* grouped within a single clade. REA put forth reciprocal monophyly (Moritz, 1994*b*) as the sole criteria for accepting divergence among subspecies; however, given the expectation of incomplete lineage sorting, this requirement was overly stringent, and it being the sole criteria for acceptance of divergence increased the likelihood that REA would conclude that no differences exist among subspecies. Notably, and consistent with an understanding that incomplete lineage sorting can complicate the understanding of phylogenetic history, Moritz (1994*a*) modified his proposal of reciprocal monophyly with the suggestion that significant, but not necessarily absolute, separation of alleles among populations is an appropriate indicator of the presence of distinct, taxonomically recognizable entities.

Although we find the current phylogeny generated by REA to be preliminary, the marked differences in haplotype frequencies observed among the five subspecies clearly support divergence. In order to further explore the pattern of haplotype frequencies among the different subspecies, we designated each observed haplotype (from REA) to the subspecies within which it occurred with the highest frequency (calculated from Appendix 2 of REA); for example, all L and L/PAL haplotypes were assigned as '*luteus* haplotypes' (with frequencies in *Z. h. luteus* of 1.00), although they also occur in *Z. h. pallidus* and *Z. h. campestris* at much lower frequencies (0.059 and 0.129, respectively; Table 1). 'Contaminant' haplotypes may result from incomplete lineage sorting, migration from adjacent subspecies or misidentification of individuals at subspecific boundaries. Although both incomplete lineage sorting and migration of individuals from adjacent subspecies would be expected, other cases of supposed 'contamination' more likely result from misidentification of individuals. For example (Appendix 2 of REA), three individuals of '*Z. h. intermedius*' from Harding Co. in north-western South Dakota (Fig. 1) with the C5/INT13 haplotype (designated as a '*campestris* haplotype') are mapped by REA (their fig. 4) as occurring within the range of *Z. h. campestris*, and two individuals of '*Z. h. pallidus*' from Clay Co. in extreme south-eastern South Dakota (Fig. 1) with the PAL1/INT15 haplotype (designated as an '*intermedius* haplotype') are the only '*Z. h. pallidus*' found within the range of *Z. h. intermedius*, north of the Missouri River. Even if we assume these individuals were correctly assigned to subspecies, *Z. h. preblei, Z. h. luteus, Z. h. intermedius* and *Z. h. pallidus* exhibited low frequencies of 'contaminant' haplotypes of all types, whereas *Z. h. campestris* contained an admixture of haplotypes (Table 1, Fig. 1).

Table 1 Frequency of subspecies characteristic haplotypes (assigned to subspecies based on highest frequency of occurrence) within five subspecies of *Zapus hudsonius*

	Subspecies				
Haplotype	*Preblei*	*Luteus*	*Intermedius*	*Pallidus*	*Campestris*
Preblei	**1.000**				0.226
Luteus		**1.000**		0.059	0.129
Intermedius			**0.915**	0.059	0.258
Pallidus			0.021	**0.882**	
Campestris			0.064		**0.387**

The frequency at which each subspecific haplotype is found within each subspecies is shown in boldface along the diagonal; squares indicate ancestral haplotypes shared likely due to incomplete lineage sorting; ovals indicate results of possible migration or mistaken subspecific identification (based on geographic location)

The unique admixture of haplotypes in *Z. h. campestris* may indicate a previously more widespread distribution (allowing retention of ancestral haplotypes), may simply reflect that subspecies' geographic position adjacent to three other subspecies (opportunities for migration and misidentification), or a combination of both factors. Notably, no contaminant haplotypes were found in *Z. h. preblei*, and although '*preblei* haplotypes' were also found in the highly admixed *Z. h. campestris*, the haplotype frequency differences between these subspecies were striking (Fig 1, Table 1 and REA fig. 3). This pattern of significant haplotype frequency differences occurring in conjunction with a lack of reciprocal monophyly for two closely related lineages is consistent with the process of incomplete lineage sorting wherein ancestral polymorphism of haplotypes is retained across divergent lineages at low frequencies (Avise, 2000). Such incomplete sorting of haplotypes is not only expected theoretically, but has also been well documented in a wide

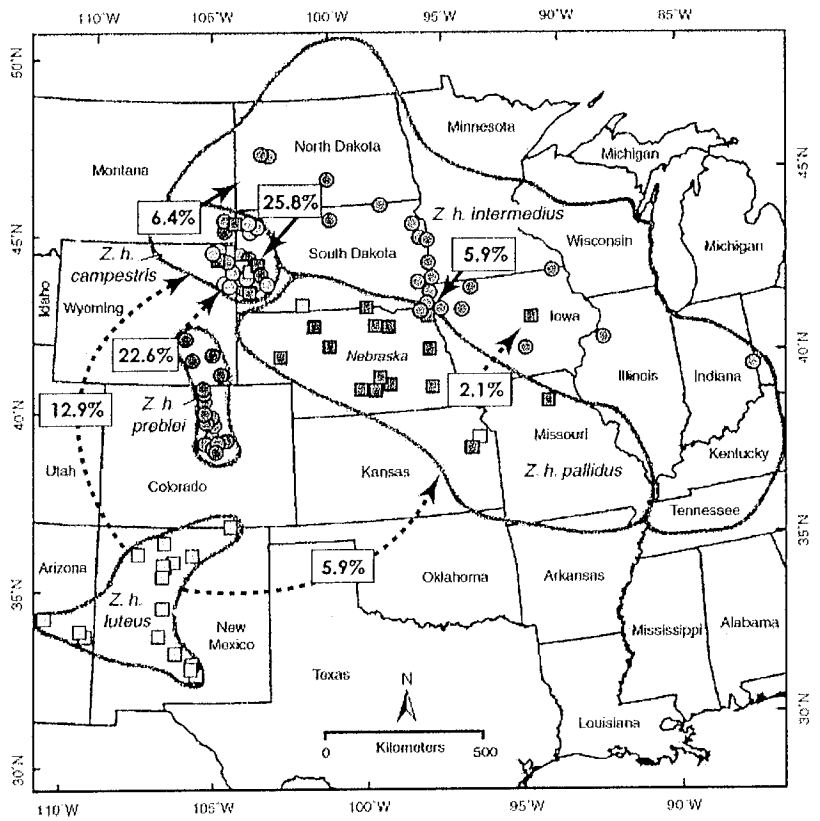

Figure 1 Distribution of mtDNA haplotypes among five subspecies of *Zapus hudsonius*. Squares = *pallidus-luteus* lineage, circles = *intermedius-campestris-preblei* lineage (from fig. 3 of Ramey *et al.* 2005). Colors (modified from the original figure) indicate haplotype assignment to subspecies (see text and Table 1). Percentages of haplotypes characteristic of one subspecies and found in another are indicated within boxes next to arrows. Solid arrows indicate probable migration or mistaken subspecific identification of samples; dotted arrrows indicate probable shared-ancestral haplotypes due to incomplete lineage sorting

variety of organisms, including taxa that are clearly separate biological species (Avise, 2000).

Given the availability of rapid DNA sequencing technology, universal primers for mtDNA amplification and numerous nuclear loci for mammals (48 reported by Yang & Nielsen, 1998), the short sequence of the single mtDNA locus used by REA represents a minimal effort toward revealing patterns of divergence in this group and should be observed as only a preliminary foray into its true evolutionary history. Many studies investigating similar questions of lineage divergence have used much higher standards and these should be viewed as more solid models for taxonomic investigation. For example, Roca *et al.* (2001) used 1732 bp from four nuclear DNA genes to separate African forest elephants from savannah elephants as separate species. Culver *et al.* (2000) used 891 bp of mitochondrial DNA and 10 DNA microsatellites to collapse 15 historically recognized subspecies of puma into six subspecies, and Jones *et al.* (in press) used 1900 bp of combined mitochondrial and nuclear DNA sequences and 10 DNA microsatellites to distinguish populations of endangered freshwater mussels as either species or subspecies. These studies also used geography, life history, behavior and morphology to corroborate their findings. Given the strength of the arguments for the use of multiple loci in phylogenetic studies and the prevalence of numerous studies demonstrating much higher standards of data inclusion, the single-locus, short sequence approach used by REA should be viewed as precursory and most certainly should not be presented as an adequate basis for the making of taxonomic decisions regarding a listed taxon.

The taxa investigated by REA clearly violate an assumption of the MDIV test for gene flow among subspecies, the assumption of equal effective population size (N_e). Nevertheless, if we assume their estimates are generally accurate, the degree of gene flow between *Z. h. preblei* and *Z. h. campestris* is very low, an unscaled rate of 0.000033 to 0.0000032 individuals per generation. This rate does not qualify as homogenizing gene flow. Natural hybridization among well-differentiated species can occur at rates higher than this (e.g. Campton & Utter, 1985; Arnold, 1992; Roques, Sevigny & Bernatchez, 2001), and low levels of gene flow do not preclude local adaptation (Broggi *et al.*, 2005). Although complete introgressive hybridization

(i.e. hybrid swarms) may exclude hybridized populations from the units considered for listing under the US-ESA (Allendorf *et al.*, 2004), REA quite clearly demonstrate that this level of introgression is *not* occurring among *Z. h. preblei* and other subspecies.

Microsatellites

Similar to the analysis of mtDNA sequence data, REA used too few loci in the microsatellite analysis to ensure high resolution. Smouse & Chevillon (1998) state that 'large numbers of polymorphic loci' are required 'to assign individuals to their correct population' and emphasize that there is a positive relationship between the number of populations in question and the number of loci required to place individuals correctly. In initially describing the STRUCTURE method used by REA, Pritchard, Stephens & Donnelly (2000) were unable to acquire a clear estimate for K (the number of populations represented within the sample) with their simulated dataset using five polymorphic loci. Further, they concluded that 'the accuracy of assignment depends on. . .the number of loci [which will affect the accuracy of q_{MAX} (likelihood of assignment of an individual to a given cluster)].' Although locus availability is often a problem, as of 2003 there were at least eight additional microsatellite loci for *Zapus* spp. (Vignieri, 2003) available for use by REA.

Given the expected low resolving power of the microsatellite data, REA's results are surprisingly strong in support of differentiation of *Z. h. preblei* from the other subspecies. F_{ST} values that are significantly different from zero indicate that gene flow among the compared populations is limited enough to result in genetic divergence. F_{ST} values observed among *Z. hudsonius* subspecies were significant for all pairwise comparisons, indicating that variation in allele frequencies among subspecies was greater than that within subspecies (Wright, 1951; Weir & Cockerham, 1984); thus the subspecies are genetically diverged. Although REA argue that their observed F_{ST} values are low (0.07–0.16), they are well within the range generally observed among subspecies in mammals (gray wolf 0.168, Roy *et al.*, 1994; African buffalo 0.059, Van Hooft, Groen & Prins, 2000; jaguar 0.065, Eizirik *et al.*, 2001). Further, REA report high per-locus polymorphism and high values of within-population heterozygosity, H_s (0.69–0.94). Considering the value of F_{ST} can be no larger than $1-H_s$ (Hedrick, 1999), even with complete differentiation, the highest absolute F_{ST} we would expect for the loci used by REA ranges from 0.06 to 0.31, and thus the F_{ST} values observed among subspecies are relatively high.

Strong support for differentiation among subspecies is also found in the STRUCTURE analysis. Although resolving power with five loci is limited, q_{MAX} for both *Z. h. preblei* populations was quite high ($q_{MAX} = 0.85$ for the northern population and 0.86 for the southern population). All other subspecies had lower q_{MAX} values, including *Z. h. luteus* (0.67), whose distinctiveness REA do not question. Similarly, correct assignment proportions for both northern (42.9%) and southern (54.5%) populations of *Z. h. preblei* were considerably higher than those observed in any other subspecies, including *Z. h. luteus* (only 21.9% of individuals correctly assigned). Additionally, 95% of the northern population and 94% of the southern population of *Z. h. preblei* were assigned to two clusters (2 and 5) that had very few individuals assigned from any of the other subspecies (REA table 6). Given the low resolving power of the loci used by REA, the relatively high proportion of correct assignment observed in *Z. h. preblei* populations provides further strong evidence of differentiation.

Use and Interpretation of AMOVA

REA used AMOVA as a measure of distinctiveness of *Z. h. preblei*, and set the criterion that there must 'be greater molecular variance among than within subspecies.' Results from mtDNA sequences showed that 18.5–37% of variation was found between subspecies, and microsatellite data indicate that 7.5–9% of variation occurred between populations. Although the authors do not present a significance value for the AMOVA test, they claim that *Z. h. preblei* fails these tests of genetic uniqueness. However, the within-population component of total genetic diversity may exceed the between-population component even when comparing separate species. For example, Leibers, Helbig & De Knijff (2001; using mtDNA sequence data) found that only 26.8% of the total diversity among gull populations resides among acknowledged species. Using microsatellite data, Grobler *et al.* (2005) found that only 29.2% of the total variation among blue and black wildebeest populations occurs between species. Thus, it is not necessarily expected that an AMOVA-based analysis of subspecies, or even species, will reveal more diversity among than within subspecies. The criterion used by REA was dubious at best, and the conclusion drawn from failure to meet this criterion is not valid.

Conclusion

The definition of taxonomic groups has long been an area of contention. Species concepts are abundant and continuously debated (a recent count listed 24; Mayden, 1997), and concepts of subspecies are even less well defined. Given the uncertainty present in both the definition of taxonomic status and the identification of such, in our efforts to preserve biodiversity we should be striving to protect populations of organisms that are on separate evolutionary trajectories rather than debating taxonomic definitions (Waples, 1991; Hey *et al.*, 2003). This desire has been expressed in both the literature and the intent of government policy where evolutionarily significant units (ESUs) and distinctive population segments (DPSs) have been identified as groups worthy of protection. Within the US-ESA, species are defined as 'any subspecies of fish or wildlife or plants, and any *distinct population segment* of any species of vertebrate fish or wildlife which interbreeds when mature' [16 U.S.C., Sec. 1532(16)] and it states that the definition of such groups should be determined based 'solely on the best

available science.' Clearly, the intent of conservation policy is to protect populations identified, in a scientifically rigorous way, as evolutionarily distinctive. Given the clarity of this intention, we find REA's recommendation of synonymy of *Z. h. preblei* curious and unjustified.

We firmly believe that no single approach should be used as a 'taxonomic litmus test' for taxa of conservation concern. However, for cases where such testing is appropriate, we offer a simple alternative hypothesis-testing approach based on the understanding that conservation of biodiversity requires conservation of groups that are evolutionarily distinct. Given this goal, we can address questions of conservation units based on this null hypothesis: These populations of individuals represent a readily interbreeding, undifferentiated unit with shared adaptations and a common evolutionary trajectory. What we are truly interested in revealing is whether there is *any* evidence that a given group is evolutionarily unique and therefore an important component of global biodiversity. Considering the data on *Z. hudsonius* subspecies presented by REA and other published information on the taxa and their environments we have discussed, we find the null hypothesis, that this group represents one readily interbreeding, undifferentiated unit, can be rejected, and the alternate hypothesis, that the populations currently classified as subspecies represent unique evolutionary entities, can be accepted across *all* of REA's informative lines of evidence. Gene flow between these disjunct subspecies is exceedingly low; there is evidence that *Z. h. preblei* is diverged in morphology and strong evidence that it is substantially diverged in mtDNA haplotype frequencies and microsatellite allele frequencies and allelic distribution.

Because REA assert a challenge to the Preble's meadow jumping mouse's current classification as a subspecies, the burden of proof is upon them to provide clear, solid evidence that this taxon is not evolutionarily distinct and thus its subspecific classification is unwarranted. Contrary to REA's stated conclusions, we find no evidence supporting their extreme recommendation of synonymy and instead conclude that their evidence offers further support for the classification of *Z. h. preblei* as a unique subspecies and a distinct evolutionary unit worthy of the protection it is currently afforded. Finally, we caution that vague questions of 'taxonomic validity' can undermine the intent to protect evolutionarily distinct units and we urge that this study not be considered a precedent for evaluation of validity in taxa of conservation concern.

References

Allendorf, F.W., Leary, R.F., Hitt, N.P., Knudsen, K.L., Lundquist, L.L. & Spruell, P. (2004). Intercrosses and the US Endangered Species Act: should hybridized populations be included as westslope cutthroat trout? *Conserv Biol.* **18**, 1203–1213.

Arbogast, B.S., Edwards, S.V., Wakeley, J., Beerli, P. & Slowinski, J.B. (2002). Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annu. Rev. Ecol. Syst.* **33**, 707–740.

Arnold, M.L. (1992). Natural hybridization as an evolutionary process. *Annu. Rev. Ecol. Syst.* **23**, 237–261.

Avise, J.C. (2000). *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.

Bain, M.R. & Shenk, T.M. (2002). Nests of Preble's meadow jumping mouse (*Zapus hudsonius preblei*) in Douglas County, Colorado. *Southwest. Nat.* **47**, 630–633.

Barratt, E.M., Deaville, R., Burland, T.M., Bruford, M.W., Jones, G., Racey, P.A., & Wayne, R.K. (1997). DNA answers the call of pipistrelle bat species. *Nature* **387**, 138–139.

Broggi, J., Hohtola, E., Orell, M. & Nilsson, J.A. (2005). Local adaptation to winter conditions in a passerine spreading north: a common-garden approach. *Evolution* **59**, 1600–1603.

Brook, A., Zint, M. & De Young, R. (2003). Landowners' responses to an Endangered Species Act listing and implications for encouraging conservation. *Conserv. Biol.* **17**, 1638–1649.

Campton, D.E. & Utter, F.M. (1985). Natural hybridization between steelhead trout (*Salmo-gairdneri*) and coastal cutthroat trout (*Salmo-clarki-clarki*) in 2 Puget sound streams. *Can. J. Fish. Aqua. Sci.* **42**, 110–119.

Chapman, S.S., Bryce, S.A., Omernik, J.M., Despain, D.G., ZumBerge, J. & Conrad, M. (2004). *Ecoregions of Wyoming, color poster with map, descriptive text, summary tables, and photographs*. Reston, VA: United States Geological Survey.

Conner, M.M. & Shenk, T.M. (2003). Distinguishing *Zapus hudsonius preblei* from *Zapus princeps princeps* by using repeated cranial measurements. *J. Mammal.* **84**, 1456–1463.

Cryan, P.M. (2004). *Synthesis of existing information on meadow jumping mice (Zapus hudsonius) in the northern great plains*. An informative review compiled for the U.S. Fish and Wildlife Service, Region 6, Denver, CO: 1–27.

Culver, M., Johnson, W.E., Pecon-Slattery, J. & O'Brien, S.J. (2000). Genomic ancestry of the American puma (*Puma concolor*). *J. Hered.* **91**, 186–197.

Edwards, S.V. & Beerli, P. (2000). Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* **54**, 1839–1854.

Eizirik, E., Kim, J.H., Menotti-Raymond, M., Crawshaw, P.G., O'Brien, S.J. & Johnson, W.E. (2001). Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). Mol. Ecol. **10**, 65–79.

Gift, N. & Stevens, P.F. (1997). Vagaries in the delimitation of character states in quantitative variation – an experimental study. *Syst. Biol.* **46**, 112–125.

Grobler, J.P., Hartl, G.B., Grobler, N., Kotze, A., Botha, K. & Tiedeman, R. (2005). The genetic status of an isolated black wildebeest (*Connochaetes gnou*) population from the

Abe Bailey Nature Reserve, South Africa: microsatellite data on a putative past hybridization with blue wildebeest (*C. taurinus*). *Mammal. Biol.* **70**, 35–45.

Hedrick, P.W. (1999). Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution* **53**, 313–318.

Hey, J., Waples, R.S., Arnold, M.L., Butlin, R.K. & Harrison, R.G. (2003). Understanding and confronting species uncertainty in biology and conservation. *Trends Ecol. Evol.* **18**, 597.

Krutzsch, P.H. (1954). North American jumping mice (genus *Zapus*). *Univ. Kansas Publ. Mus. Nat. Hist.* **7**, 349–472.

Küchler, A.W. (1970). Potential natural vegetation. In *The national atlas of the United States of America*, ed. United States Department of the Interior, Geological Survey, 89–92. Washington, D.C.: United States Government Printing Office.

Leibers, D., Helbig, A.J. & De Knijff, P. (2001). Genetic differentiation and phylogeography of gulls in the *Larus cachinnans-fuscus* groups (Aves: Charadriiformes). *Mol. Ecol.* **10**, 2447–2462.

Maddison, W.P. (1997). Gene trees in species trees. *Syst. Biol.* **46**, 523–536.

Mayden, R.L. (1997). A hierarchy of species concepts: the denouement in the saga of the species problem. In *Species: the units of biodiversity*: 381–424. Claridge, M.F. (Ed.). London: Chapman & Hall.

Meaney, C.A., Ruggles, A.K., Lubow, B.C. & Clippinger, N.W. (2003). Abundance, survival, and hibernation of Preble's meadow jumping mice (*Zapus hudsonius preblei*) in Boulder County, Colorado. *Southwest. Nat.* **48**, 610–623.

Minitab I. (1996). *Minitab reference manual*. State College, PA: Minitab, Inc.

Moritz, C. (1994*a*). Applications of mitochondrial-DNA analysis in conservation – a critical review. *Mol. Ecol.* **3**, 401–411.

Moritz, C. (1994*b*). Defining evolutionarily-significant units for conservation. *Trends Ecol. Evol.* **9**, 373–375.

O'Brien, S.J. & Mayr, E. (1991). Bureaucratic mischief – recognizing endangered species and subspecies. *Science* **251**, 1187–1188.

Poe, S. & Wiens, J.J. (2000). Character selection and the methodology of morphological phylogenetics. In *Phylogenetic analysis of morphological data*: 20–36. Wiens, J.J. (Ed.). Washington, DC: Smithsonian Institution Press.

Poole, W.E., Carpenter, S.M. & Simms, N.G. (1980). Multivariate analyses of skull morphometrics from the two species of grey kangaroos, *Macropus giganteus* Shaw and *M. fuliginosus* (Desmarest). *Aust. J. Zool.* **69**, 591–605.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Ramey, R.R. II, Liu, H.P., Epps, C.W., Carpenter, L.M. & Wehausen, J.D. (2005). Genetic relatedness of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA and microsatellite DNA: implications for taxonomy and conservation. *Anim. Conserv.* **8**, 329–346.

Roca, A.L., Georgiadis, N., Pecon-Slattery, J. & O'Brien, S.J. (2001). Genetic evidence for two species of elephant in Africa. *Science* **293**, 1473–1477.

Roques, S., Sevigny, J.M. & Bernatchez, L. (2001). Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the north-west Atlantic: a rare marine example. *Mol. Ecol.* **10**, 149–165.

Roy, M.S., Geffen, E., Smith, D., Ostrander, E.A. & Wayne, R.K. (1994). Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. *Mol. Biol. Evol.* **11**, 553–570.

Schorr, R.A. & Davies, R. (2002). Grey flesh fly (*Wohlfahrtia vigil*) parasitism of a Preble's meadow jumping mouse (*Zapus hudsonius preblei*). *J. Wildl. Dis.* **38**, 604–606.

Smouse, P.E. & Chevillon, C. (1998). Analytical aspects of population-specific DNA fingerprinting for individuals. *J. Hered.* **89**, 143–150.

USFWS (2004). *Preble's meadow jumping mouse recovery plans*. USFWS Mountain-Prarie Region Endangered Species Program.

Van Hooft, W.F., Groen, A.F. & Prins, H.H.T. (2000). Microsatellite analysis of genetic diversity in African buffalo (*Syncerus caffer*) populations throughout Africa. *Mol. Ecol.* **9**, 2017–2025.

Vignieri, S.N. (2003). Isolation and characterization of eight highly variable microsatellite markers in the Pacific jumping mouse (*Zapus trinotatus*). *Mol. Ecol. Notes* **3**, 638–640.

Wakeley, J. (2000). The effects of subdivision on the genetic divergence of populations and species. *Evolution* **54**, 1092–1101.

Wakeley, J. (2001). The coalescent in an island model of population subdivision with variation among demes. *Theor. Popul. Biol.* **59**, 133–144.

Waples, R.S. (1991). Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. *Mar. Fish. Rev.* **53**, 11–22.

Weir, B.S. & Cockerham, C.C. (1984). Estimating F-statistics for the analysis of population–structure. *Evolution* **38**, 1358–1370.

Whitaker, J.O. (1970). The biological subspecies: an adjunct of the biological species. *Biologist* **52**, 12–15.

Wright, S. (1951). The genetical structure of populations. *Ann. Eugenics* **15**, 323–354.

Yang, Z.H. & Nielsen, R. (1998). Synonymous and nonsynonymous rate variation in nuclear genes of mammals. *J. Mol. Evol* **46**, 409–418.

Zink, R.M. (1988). Evolution of brown towhees: allozymes, morphometrics and species limits. *Condor* **90**, 72–82.

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Re: Proposed Delisting of the Preble's Meadow Jumping Mouse

To Whom It May Concern:

The Wyoming Contractors' Association (WCA) is an organization promoting, protecting, and serving the Wyoming construction industry. Since our establishment in 1932, the construction industry has drastically evolved and has faced more complex issues and challenges. The construction industry faces challenges in a wide-array of areas. Environmental issues, including endangered species issues in Wyoming, are a high priority for WCA and its 231 members.

Our members are involved in highway, heavy, building, municipal and utility construction projects. The mission of the WCA is to provide benefits and services to our members through political involvement, training, safety programs and career opportunities which offer advancement for member employees, promotes the construction industry and ensures a competitive opportunity that benefits all citizens of Wyoming.

Continued economic development is vital to Wyoming and its citizens. Carrying through with the delisting of the Preble's Mouse as originally intended will ensure that the critical habitat designations throughout Wyoming will not hinder or interfere with economic growth. Delisting a species that has not been conclusively identified, collected or found in areas of critical habitat should be the highest priority of the U.S. Fish and Wildlife Service.

Preble's Habitat

The WCA's main concern regarding the delisting of the Preble's mouse is the actual determination that such species exists within areas designated as critical habitat in Wyoming. While the delay in the delisting process stems from inconsistent scientific studies regarding the Preble's mouse, this one large issue still remains.

The Preble's mouse has been listed under the Endangered Specifies Act (ESA), since 1998, yet the two reports referenced in the latest request for comments (Ramey et al. 2004a, 2004b, 2005; King et al. 2006) challenge the existence and appropriate classification of the species. While the scientists continue to debate and issue conflicting reports, land use restrictions related to the critical habitat designation remain and have limited Wyoming economic development in those areas.

The Preble's mouse should never have been listed without more concrete data and findings. Amid all the reports and studies conducted by the USFWS and independent reviewers, there is still no conclusive determination regarding the existence of a separate subspecies or the appropriateness of its critical habitat designation in Wyoming.

After eight years of critical habitat and ESA protections, none of the reports indicate any success in the enhancement of the species. Since the Preble's mouse cannot be accurately identified, any habitat protections cannot show any results in species improvement. Without being able to identify the species and its population, how will the USFWS ever be able to determine when delisting is warranted?

The Delisting Process

After a year long study and commitment to delist the Preble's mouse, the USFWS continues to fully protect the Preble's mouse under the Endangered Species Act. The Preble's mouse is a prime example of the failures and shortcomings of the Endangered Species Act.

The Preble's mouse was listed before anyone could conclusively determine its existence as a separate sub-species. Even though a decision is expected in August, the limitations on private land use remain in those areas designated as critical habitat. Construction and development projects must also continue to protect the mouse habitat as well as apply for incidental take permits and provide additional habitat for the mouse on any project located within the areas designated as critical habitat. We urge the USFWS to look at the Preble's mouse as a prime example for the need of ESA reform and revisions. Extending a delisting decision that was originally scheduled to occur in 2005 only hampers economic development that is vital to the WCA and the State of Wyoming.

The 2005 delisting was delayed based on the USFWS's desire to have a "complete review" of *Campestris*. This was an unfair postponement and was designed to once again delay the delisting of the Preble's mouse. If the Preble's mouseis a separate sub-species, it should have been treated that way. The USFWS decision to delist should have been based on the Preble's mouse and not additional reports of the *Camapestris*.

Economic Impacts and Restrictions

Since designation of critical habitat, all aspects of development, construction, and economic growth have been halted or made much more expensive in those areas. This tiny, unidentifiable mouse has halted development on the Colorado Front Range and in Wyoming, even though there has never been a verified finding of a Preble's mouse in Wyoming. Local counties and cities have prepared habitat conservation plans, incidental take permit measures and mitigation plans in order to develop lands with Preble's mouse habitat. Eleven Habitat Conservation Plans in Colorado have been approved by the USFWS for the incidental take of the Preble's mouse and protection of its habitat. However, where is the proof that such plans are working or protecting the Preble's mouse?

Construction projects and housing developments often have to prepare mitigation measures covering areas larger than the project itself. Our industry is frustrated that such land use restrictions are in place when the USFWS has not been able to determine if the Preble's mouse is a species qualified to be listed in the first place. Restricting economic development for eight years without an actual determination of the species and its viability should alarm the USFWS about the ESA failures.

Conclusion

Restricting construction, development, and agriculture in southeastern Wyoming and along the Colorado Front Range has taken place since 1998 and citizens deserve a resolution of the issues related to this species.

There are currently more scientific studies on the Preble's ouse than when it was listed in 1998. Yet the USFWS still believes this information is not enough to warrant delisting. When will it ever be enough? Twenty-eight substantive comments were submitted to the USFWS regarding the proposed rule to delist in 2005. Many of them urged a delisting, yet the USFWS has delayed its final decision.

The USFWS should not further delay the delisting of the Preble's mouse. The WCA supports a speedy and efficient delisting of the Preble's mouse. Construction projects and undue land use restrictions have been in place for eight years without a final determination if the Preble's mouse listing is even warranted. The WCA also urges the USFWS to consider additional measures for ESA reform. We urge that delisting a species becomes as efficient as simple as listing a species for future ESA reform measures.

Sincerely,

Jonathan E. Downing
Assistant Vice President

RECEIVED
MAY 16 2006

EL PASO COUNTY
OFFICE OF THE BOARD
OF
COUNTY COMMISSIONERS
27 EAST VERMIJO AVENUE
COLORADO SPRINGS, COLORADO 80903-2208
(719) 520-7276 Fax: (719) 520-6397
Web Address: www.elpasoco.com

COMMISSIONERS

Sallie Clark	District 3 (Board Chair)
Dennis Hisey	District 4 (Vice Chair)
Jim Bensberg	District 5
Wayne Williams	District 1
Douglas Bruce	District 2

05412-2006P-0052

May 1, 2006

Ms. Susan Linner
Field Supervisor
Colorado Fish and Wildlife Office
Ecological Services
USFWS, ES, Colorado Field Office
P.O. Box 25486, DFC (MS 65412)
Denver, CO 80225

Dear Ms. Linner:

RE: Comments on the six-month extension and reopening of the comment period on the proposal to delist the Preble's Meadow Jumping Mouse (PMJM) in Colorado and Wyoming (Fed. Reg. / Vol. 71, No. 33 / Friday, February 17, 2006)

On June 1, 2004 the El Paso County Commissioners provided official comment on the 90-day finding for a petition to delist the Preble's meadow jumping mouse in Colorado and Wyoming and Initiation of a 5-year Review (Fed. Reg./ Vol. 69, No. 62/Wednesday March 31, 2004) supporting the delisting of the Preble's Mouse. The Board of County Commissioners hereby incorporates those comments herein by this reference. Please consider this letter and the attached resolution as El Paso County's additional official comments in response to the February 17, 2006, six-month extension of the proposed delisting of the PMJM in Colorado and Wyoming.

The Board of County Commissioners of El Paso County (Board) encourages the U.S. Fish and Wildlife Service (Service) to expedite review of all information related to the delisting of the PMJM. We recognize that genetic analysis is only one determinative factor, and that morphology and behavioral characteristics can also inform the question of taxonomy. We nonetheless urge the Service to carefully consider the analysis and conclusions presented in both Dr. Rob Roy Ramey's and Dr. Tim King's genetics reports, and the resulting scientific peer reviews, as well as morphological and behavioral characteristics when deciding whether to delist the PMJM. Based on this information, the Board continues to support a decision to delist the PMJM.

Since 1998, El Paso County has actively pursued the development of a Regional Habitat Conservation Plan (RHCP) for the PMJM. Since that time, El Paso County has suffered numerous setbacks in the completion of the RHCP. The 6-month extension regarding the delisting proposal creates yet another setback to the completion of this document. Additional setbacks have included the proposed critical habitat designation, the proposed recovery plan, and the initial delisting proposal. Further delays associated with the delisting decision create uncertainty for the RHCP process and the citizens of El Paso County.

In order to potentially save the County, its partners, and the citizens additional time and money, we, the El Paso County Board of Commissioners, strongly urge the Service to expedite a **FINAL** decision on the proposal to delist the Preble's meadow jumping mouse as soon as possible.

Very truly yours,

Sallie Clark

Sallie Clark, Chair
Board of County Commissioners of El Paso County

Enclosure

c: Mike Bonar, Natural Resources Manager
M. Cole Emmons, Assistant County Attorney
Kirsta Scherff-Norris, Colorado Springs Utilities
Lisa Ross, P.E., City of Colorado Springs

Office of County Clerk and Recorder
El Paso County, State of Colorado
Certified to be a full, true and Correct
Copy of record in my Office.
..... Book..... Page ....
Date 5-11-06 Robert C. Balink
County Clerk & Recorder
El Paso County, Colorado
By Kimberly D. Musselwhite
Deputy

RESOLUTION NO. 06-132

BOARD OF COUNTY COMISSIONERS
COUNTY OF EL PASO, STATE OF COLORADO

RESOLUTION TO APPROVE COMMENT LETTER TO THE U.S. FISH AND WILDLIFE SERVICE SUPPORTING THE PROPOSED DELISTING OF THE PREBLE'S MEADOW JUMPING MOUSE

WHEREAS, the Board of County Commissioners of the County of El Paso ("Board") has the authority granted to it under the provisions of Section 30-11-107(1)(e), C.R.S., to represent the County and have the care of the management of the business and concerns of the County in all cases where no other provisions are made by law; and

WHEREAS, on May 13, 1998, the United States Fish & Wildlife Service ("Service") listed the Preble's Meadow Jumping Mouse ("PMJM") as a threatened species under the Endangered Species Act; and

WHEREAS, since 1998, El Paso County, along with the City of Colorado Springs, Colorado Springs Utilities, the Town of Palmer Lake, and the Town of Monument have worked diligently to develop a Regional Habitat Conservation Plan (RHCP); and

WHEREAS, listing of the PMJM has required local government agencies and private owners to incur a great deal of expense including, but not limited to, staff and consultant time, money, education, conservation efforts, trapping and data collecting in order to comply with the Service's requirements under the Endangered Species Act; and

WHEREAS, genetic analysis is only one determinative factor, and morphology and behavioral characteristics can also inform the question of taxonomy. We urge the Service to carefully consider the analysis and conclusions presented in both Dr. Rob Roy Ramey's and Dr. Tim King's genetics reports, and the resulting scientific peer reviews, as well as morphological and behavioral characteristics when deciding whether to delist the PMJM. Based on this information, the Board continues to support a decision to delist the PMJM; and

WHEREAS, on February 17, 2006, the Service published in the *Federal Register* (Vol. 71 FR 33, pg. 8556) a notice extending for six months its decision on the proposed delisting of the PMJM in Colorado and Wyoming and reopening the public comment period; and

WHEREAS, the County through its various departments, as well as private landowners in the County and others doing business in the County may have, or do have, future and/or ongoing projects that would benefit by the delisting of PMJM; and

ROBERT C. "BOB" BALINK El Paso County, CO
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WHEREAS, further delaying the decision of whether to delist the PMJM adversely affects public health, safety, and welfare in terms of increasing completion times and expense of public project; and

WHEREAS, the Board desires to support delisting of the PMJM and, in furtherance of the delisting, approves a comment letter supporting the delisting of the PMJM to be sent to the Service.

NOW, THEREFORE, BE IT RESOLVED that the Board of County Commissioners of the County of El Paso hereby endorses the formal comments attached hereto as Exhibit A and incorporated herein by this reference, and directs that said formal comments be filed with the Service.

BE IT FURTHER RESOLVED that Sallie Clark, duly elected, qualified, member and Chair or Dennis Hisey, duly elected, qualified, member and Vice Chair of the Board of County Commissioners is hereby authorized to execute this Resolution and an original of comments in the same form as those contained in Exhibit A attached hereto on behalf of the Board of County Commissioners.

DONE THIS 1st day of May 2006, at Colorado Springs, Colorado.

BOARD OF COUNTY COMMISSIONERS
OF EL PASO COUNTY, COLORADO

By: Sallie Clark
Sallie Clark, Chair

ATTEST:

By:
Deputy Clerk to the Board

65412-2006-P0063
RECEIVED
MAY 17 2006

May 16, 2006

Via Electronic and Overnight Mail USPS 5/14/06

Susan Linner, Field Supervisor
Colorado Field Office
Ecological Services
P.O. Box 25486
Denver Federal Center
Denver, CO 80225

Re: Reopening of the Public Comment Period for the Proposed Delisting of the Preble's Meadow Jumping Mouse, 71 Fed. Reg. 8556 (February 17, 2006)

Dear Ms. Linner:

On behalf of the Natural Resources Defense Council ("NRDC") and our over 650,000 members, 20,000 of whom live in Colorado, I submit the following comments in response to the United States Fish and Wildlife Service's ("Service") proposal to delist the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) from the Endangered Species Act's ("ESA" or "the Act") list of threatened and endangered species. *See* "12-Month Finding on a Petition To Delist the Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*) and Proposed Delisting of the Preble's Meadow Jumping Mouse," 70 Fed. Reg. 5404 (February 2, 2005).

We believe that the best available scientific data does not support the Service's proposal to delist the Preble's meadow jumping mouse and that it, in fact, confirms the subspecific status of the mouse. *Z. h. preblei* has been recognized as a subspecies by mammalogists for half a century and the sole study on which the Service relied to justify the proposed delisting, Ramey et al. 2005, is methodologically flawed and its conclusions are not supported by the data. Additionally, the more recent genetic study by King et al. (2006), which quadrupled the amount of genetic information used in the Ramey et al. study, clearly confirms the mouse's subspecific status. The differences between these studies are straightforward and not a matter of interpretation. Furthermore, the patterns of genetic distinction revealed by the King et al. study are well above population level differences and even exceed some species level descriptions.

Recent peer reviews that compare the King et al. study with Ramey et al. are nearly unanimous in their support for retaining protected status for *Z. h. preblei* as a threatened subspecies[1] and NRDC concurs with these reviewers' conclusions. Thus, rather than present an additional thorough comparison of the two studies, my comments focus instead on addressing the few criticisms of King et al. and some of the inconsistencies between the two studies that were raised by a handful of reviewers.[2]

The most consistent criticism of the King et al. study by the peer reviewers was its sampling design, which focused on obtaining a greater number of individuals from fewer targeted areas. In comparison, Ramey et al. sampled fewer individuals from more locations. However, whereas Ramey et al. were broadly describing genetic variation in this group of mice for the first time in published literature, King et al. had the

[1] *See, e.g.*, Peer review comments from D. J. Hafner, J. E. Maldonado, M. Ashley, S. Oyler-McCance, S. M. Armstrong, B. R. Riddle, M. Douglas and K. Crandall.
[2] *See, e.g.*, Peer review comments from R. D. Bradley, K. Crandall.

advantage of narrowing their focus on a few disputed areas of interest. For example, the Ramey et al. study shows fairly clear separation of *Z. h. luteus* and *pallidus* from the remaining three subspecies. Therefore, the real question at hand surrounds whether there is genetic variation between *Z. h. preblei, campestris* and *intermedius*. In fact, since *Z. h. preblei* did not share any mitochondrial haplotypes with *intermedius*, the more pointed area of interest is whether there is genetic variation between *Z. h. preblei* and *campestris*, which Ramey et al. reported shared four mitochondrial haplotypes. This question was particularly important to answer, as five of the *Z. h. campestris* samples that shared the *preblei* haplotypes in Ramey et al. were collected from the same geographic location of Custer County, South Dakota. As other peer reviewers have pointed out, an alternative explanation for this pattern could be that the museum specimens were mislabeled or samples were contaminated in the lab.

To address these issues, King et al. sampled broadly from the field across the range of *Z. h. preblei*, with a total of 132 individuals sampled compared to 48 in Ramey et al. Of the other two subspecies, King et al. understandably and properly focused on *Z. h. campestris*, especially in the area of concern from Ramey et al's study – i.e., Custer County, South Dakota. King et al. sampled a total of 61 *Z. h. campestris* individuals from South Dakota and Wyoming compared with 30 from the Ramey et al. study. Considering the small range of *Z. h. campestris*, the King et al. sampling is likely to be representative of the entire subspecies. Finally, King et al. sampled similar numbers of *Z. h. intermedius* as Ramey et al. (n=47).

Given the extent of sampling of *Z. h. preblei* throughout its range and *Z. h. campestris* in Custer County, South Dakota, the chance that these two supbspecies share mitochondrial haplotypes is statistically improbable. For example, by taking the frequencies of the most common haplotypes, one can calculate the probability of not sampling that haplotype in the other subspecies if it were truly there. In this case, the likelihood that the most common haplotype for each subspecies (Zhp_07 (n=31), Zhc_04 (n=35)) was not detected in the other subspecies given the present sampling (132 and 61, respectively) is less than 7×10^{-9}. In fact, the likelihood of not sampling any of the five most common haplotypes of the other subspecies is less than 0.05. Since all five samples of *Z. h. campestris* from Ramey's study that shared haplotypes with *preblei* came from the same museum collection, a more likely explanation for the original report of shared haplotypes in this area would be mislabeling of museum specimens, laboratory contamination, or insufficient genetic data (346 base pairs versus 1374 in King et al.).

The mitochondrial sequence data of King et al. demonstrates nearly complete lineage sorting without a single shared haplotype between the 5 putative subspecies. Complete lineage sorting can be rare to find at the species level, much less at the subspecies level and would be highly improbable at the population level. One of the reviewers of the King et al. study, Robert D. Bradley, argues that the crux of the problem is in interpreting the degree of variation between the five groups under consideration, which he suggests comes down to a "philosophical debate" of "where you draw the line" between populations and subspecies.[3] As his main argument he states, "if you randomly picked populations that were somewhat isolated (genetically or by geographic distance) you might find evidence of little or no gene flow, especially with microsatellite data." However, Bradley ignores the fact that King et al.'s study includes not only an impressive amount of microsatellite data, but a substantial amount of mitochondrial sequence data as well. Given the relatively slowly evolving nature of mitochondrial sequence data (compared to microsatellites), the pattern revealed by the mitochondrial DNA in the King et al. study is simply not what one would expect to find between "populations that were somewhat isolated" as Bradley describes. The five subspecies have clearly been separated for a significant amount of time.

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[3] *See* Peer review comments from R. D. Bradley.

Another reviewer, Keith Crandall, incorrectly states that King et al.'s "initial finding was for three clusters, one of which was the Z. h. *preblei/intermedius/campetris* complex in question."[4] In fact, the 3 clusters identified *Z. h. preblei* as its own unique and distinct cluster (see Figure 2, King et al.). *Intermedius/campestris* formed the second cluster and *pallidus/luteus* the third. In his summary conclusions, Crandall also says that the phylogenetic trees presented in King et al. identify three clades that agree with the three clusters found by structure. Again this is not accurate. While it is true that *pallidus/luteus* form one clade and *preblei/intermedius/campetris* another, the third clade separates *intermedius/campestris* from *Z. h. prebeli* with 88-90% statistical support (see Figure Figure 7a, King et al). Therefore, both phylogenetic support from mitochondrial analyses as well as structure analyses from microsatellites in the King et al. study clearly identify *Z. h. preblei* as separate and distinct from all other subspecies.

Crandall further criticizes King et al. for not testing for ecological or genetic exchangeability between groups. Few ecological data are available for these subspecies and morphological measurements have proven to be an inconsistent surrogate. However, a lack of genetic exchangeability between these groups is supported by the absence of shared mitochondrial haplotypes and the extremely high assignment rates (>99%) at the subspecies level based on nuclear microsatellite data. While Crandall suggests that assignment is not a good verification of the results, since it is the inverse of the cluster partitioning, as stated above, he incorrectly assessed the original partitioning which clearly identifies *Z. h. preblei* as a distinct cluster. Thus, both the nuclear and the mitochondrial genomes indicate genetic uniqueness and therefore, lack of exchangeability between *Z. h. preblei* and all other subspecies.

Finally, Crandall criticizes King et al. for including too much genetic data in what he characterizes as "an overkill and fairly desperate attempt to find variation at all costs." This is simply not a valid scientific critique of the King et al. study. If genetic variation were absent from these subspecies, additional genetic sampling would only serve to confirm this pattern. In Crandall's own words, more data "are by definition, better." In this case, additional genetic sampling reveals variation that was undetected by too little data in the Ramey et al. study.

In closing, the overwhelming scientific consensus supports retaining the original taxonomic classification of *Z. h. preblei* as a threatened subspecies of the Endangered Species Act. Arguments to the contrary, including the study conducted by Ramey et al. and comments from a limited number of reviewers, are based on questionable museum samples, insufficient genetic data and a misunderstanding of the most recent study by King et al. With an improved study design, including more intensive sampling of individuals and genetic data, King et al. has unequivocally demonstrated the evolutionary distinctness of *Z. h. preblei* from all other subspecies in the region.

Thank you for considering these comments.

Sincerely,

Sylvia M. Fallon, Ph.D.
Conservation Genetics Fellow
Natural Resources Defense Council

[4] *See* Peer review comments from K. Crandall p. 5.

USFWS Colorado Field Office
134 Union Boulevard, Suite 670, Lake Plaza North
Lakewood, CO 80228
Via facsimile: 303-236-4005

May 17, 2006

COMMENTS OF THE WHEATLAND IRRIGATION DISTRICT ON THE PROPOSED DELISTING OF THE PREBLE'S MEADOW JUMPING MOUSE.

Wheatland Irrigation District ("WID")has approximately 950 members that irrigate more than 54,000 acres in Platte County, Wyoming. Lands irrigated by our members are within the historic range of the Preble's Meadow Jumping Mouse ("Preble's mouse").

Agriculture plays a vital role in Platte County by providing revenue to the local economy as well as the state of Wyoming Less than two percent of the American population are agricultural producers, yet the agricultural industry is responsible for feeding, clothing, and creating jobs for the entire state, nation and world. Any type of regulation on agriculture, regardless of the amount of area or individuals affected, is an action that has adverse impacts on the agricultural economy.

The WID appreciates the opportunity to submit these comments on the proposed delisting of the Preble's mouse. We must admit that the entire process related to the Preble's mouse (from listing to designation of critical habitat to development of a recovery plan to delisting) has left us frustrated and confused. No matter the missteps of the past, the WID believes the USFWS must take this opportunity to delist the Preble's mouse. Such a decision is consistent with the requirements of the Endangered Species Act ("ESA") and will restore credibility to the agency to continue their statutory purpose of protecting legitimate species who deserve their full attention in recovery efforts.

The WID strongly supports the delisting effort and provides the following comments to convey our experience related to the Preble's mouse.

The WID continues to believe that the USFWS did not have the scientific evidence necessary to list the Preble's mouse in May, 1998. The lack of data regarding the Preble's mouse has remained a critical issue since that initial decision that has severely impacted the USFWS reputation on this, and other species issues.

The WID became even more concerned about the listing decision when the USFWS agreed in a court-mediated settlement to designate critical habitat by June, 2003. The USFWS has admitted that designation of critical habitat under the best of circumstances "usually offers relatively little conservation benefit". Given that

admission and the method upon which designation of critical habitat for the Preble's mouse proceeded, the WID became concerned that the plaintiffs in that matter dictated the designation process more than any scientific basis that the USFWS may have used. It was also clear that since so much of the mouse's historic range in Colorado had already been developed, there would have to be an additional offset in Wyoming. While it was not legally challenged, the WID believes the process for designating 10,542 acres in Wyoming as critical habitat for the Preble's mouse was illegitimate because it was forced in a certain time-frame, without the proper amount of study and Wyoming lands were unfairly included to pay for Colorado's prior development. Needless to say, the broad designation in Wyoming has unnecessarily limited land use and further negatively impacted the credibility of the USFWS to protect truly endangered and threatened species.

A great deal of confusion has resulted from the designation of critical habitat for the Preble's mouse. For example, the USFWS continuously reminds us that the critical habitat designation has no effect when a Federal agency is not involved. However, 65% of the designated lands are privately owned and the ESA prohibits any individual from engaging in unauthorized activities that will actually harm listed species. The USFWS assurances were even harder to accept when the WID reviewed the Ditch Maintenance Guidelines developed by the USFWS for certain irrigation districts in Colorado. If these guidelines were in effect within the WID, it would vastly alter our historic operation and maintenance activities. These guidelines illustrate the difficult position of an irrigation district when its lands are part of the habitat designation, even if they are privately owned and not seeking any federal permits.

The WID Board of Directors made an early decision to play an active role in the Preble's mouse regulatory process by having me, as manager, participate in meetings regarding development of a Recovery Plan for the Preble's mouse. As you know, the Preble's Recovery Team was formed in 2000 and we completed our work by submitting recommendations in June, 2003. I participated in this effort at great expense, both in time and money, to the WID. The WID believed it was important, however, to attempt to resolve this issue by participating in the recovery team process. To say that I am disappointed that the USFWS has never approved the Preble's Recovery Plan is an understatement. A tremendous amount of energy and effort went into the development of that plan for seemingly no purpose. The USFWS said the Recovery Plan would be completed and adopted by the agency no later than December, 2003, but nearly two and one-half years later, nothing has been accomplished with the Recovery Plan. The handling of the Recovery Plan has created additional frustration and confusion about the USFWS management of the Preble's mouse.

We understand that the Recovery Plan was "shelved" because of the report issued by Dr. Ramey, who concluded that the Preble's mouse was not a separate sub-species. We welcomed Dr. Ramey's conclusions and looked forward to the delisting process. However, all that we have seen is further delay. Instead of proceeding with delisting, the USFWS is now focused on the King report, which disagrees with Dr. Ramey. In fact, the USFWS has gone so far as to commission a panel to determine whether or not the

Preble's mouse in a separate sub-species. It is somewhat unbelievable to the WID that enormous amounts of monies have been spent, and development and land use activities limited without a definitive answer to this most basic question. The WID believes that this question should have been answered *prior* to listing of the Preble's mouse. Nonetheless, it does not seem justifiable to the WID to continue land use restrictions when there does not seem to be a definitive answer to the existence question.

The fact remains that the most definitive scientific work on the Preble's mouse was completed by Dr. Ramey. Dr. Ramey has suffered tremendous personal and professional costs for stating his scientific conclusion that runs counter to the goals of environmentalists. Dr. Ramey's work has been peer reviewed and scientifically accepted. The USFWS will always be able to find other scientists to disagree with Dr. Ramey's conclusions. The WID encourages the USFWS to stop its scientific shopping excursion, accept Dr. Ramey's conclusions and delist the Preble's mouse.

Every step in the process of "protecting" the Preble's mouse has lacked credibility and defied logic. All of the factors described above contribute to the uneasiness the WID has with the entire regulatory process involving the Preble's mouse. The WID has remained involved in the issue, participated in recovery efforts and attempted to understand the process. We were elated to learn of Dr. Ramey's conclusions and applauded the USFWS delisting efforts only to be frustrated by further delays. It is time to end this debacle, delist the Preble's mouse, and lift all restrictions on lands located within the designated critical habitat.

The WID remains committed to working with the UWFWS on legitimate issues involving endangered and threatened species and hopes the USFWS will seize this opportunity to restore its reputation related to those efforts.

Thank you for your consideration,

Don Britton

Don Britton, Manager
Wheatland Irrigation District

WHEATLAND IRRIGATION DISTRICT

FACSIMILE TRANSMITTAL SHEET

TO: USFWS Colorado Field Office	FROM: Don Britton, Manager
COMPANY:	DATE: 5/17/2006
FAX NUMBER: 303-236-4005	TOTAL NO. OF PAGES INCLUDING COVER: 4
PHONE NUMBER:	WID FAX NUMBER: 307-322-1749
RE: Preble's Comment	WID PHONE NUMBER: 307-322-2740

☐ URGENT ☐ FOR REVIEW ☐ PLEASE COMMENT ☐ PLEASE REPLY ☐ PLEASE RECYCLE

NOTES/COMMENTS:

WID, PO BOX 727, WHEATLAND, WY 82201

Crystlplc@cs.com
05/17/2006 06:13 PM

To fw6_PMJM@fws.gov
cc Crystlplc@cs.com
bcc
Subject RE: Preble's Meadow Jumping mouse

What a waste of taxpayer money. Just what did it cost to do these two studies?
Now you throw more waste after the original waste. I goes without saying that
the Fish and Wildlife service should go the way of the Preble Meadow Jumping mouse, and be history.

You people should have your ass fired for shear stupidity.

With Regards,

Dale Moore

"USFWS comments"
<comments123@msn.com>
05/18/2006 10:20 AM

To <FW6_PMJM@fws.gov>
cc <Peter_Plage@fws.gov>
bcc
Subject Comments on Preble's Mouse Delisting

We are a group of concerned scientists in the fields of ecology and mammalogy.

Please accept our comments regarding the delisting of the Preble's meadow jumping mouse. We hope this comments will be of aid in the listing decision.

CommentResponseZhpreblei_ConcernScientistsMay06.pdf

Delisting of *Zapus hudsonius preblei* - Comment to U.S. Fish and Wildlife Service (70 FR 5404)

Introduction

We, the undersigned, are individuals who have worked on Preble's meadow jumping mouse (*Zapus hudsonius preblei*) in various scientific capacities. We address issues concerning genetics, ecology, and population vulnerability of the *Z. h. preblei*. In this letter, we urge the U.S. Fish and Wildlife Service not to delist *Z. h. preblei* given recent genetics research results that indicate this subspecies as genetically distinct and that populations, especially in Colorado, are still at risk and worthy of continued protection.

Genetics

Analysis of genetic variation within and among populations provides the basis for inferring whether geographically separate groups of individuals, like *Z. h. preblei* and *Zapus hudsonius campestris* (the Bear Lodge meadow jumping mouse) comprise distinct gene pools or not. Ideally, assessments of the connectedness of recognizably distinct groups rely on multiple genetic markers because any one gene can show a pattern that is anomalous due to of the effects of natural selection or hybridization. In addition, because the fate of alleles in populations is governed by stochastic processes, inferences of demographic parameters, like estimates of gene flow between groups, are uncertain. When only a single gene marker has been used when estimating gene flow, as is the case for the Ramey et al. (2005) analysis of *Zapus* subspecies, the uncertainty is especially great. In addition to the uncertainty associated with estimation of demographic parameters, sampling DNA is limited because only a small fraction of the genome is sampled; in the case of the data presented by Ramey et al, only about 300 base pairs of a maternally-inherited cytoplasmic genome were examined. The genome of the house mouse (*Mus musculus*) is 2.6 billion base pairs; assuming that the genome of *Zapus hudsonius* is approximately the same size as that of the house mouse, Ramey et al. (2005) surveyed less than 0.0001% of the genome, and only examined bits of DNA passed on through females. Finally, Ramey et al. (2005) reported that because there is evidence of recent gene flow between *Z. h. preblei* and *Z. h. campestris*, that the two subspecies should be synonymized and considered the same subspecies. However, gene flow is only a homogenizing force if the number of migrants per generation exceeds 1, and then only if there is no selection influencing the fate of migrant alleles. More realistic estimates suggest that homogenization occurs when the number of migrants per generation exceeds 5-10. When the estimated gene flow is less than 1, populations will diverge due to genetic drift and, if all things remain the same, the two populations are sufficiently isolated (genetically) for speciation to occur. The best estimate of the number of migrants per generation for the mtDNA data assembled by Ramey et al. is about 0.5, much less than the value of one or more required to homogenize population under ideal conditions. We are not advocating that the results support that there are two subspecies in nature; we are simply underscoring that the data are much too preliminary and the results too shrouded in uncertainty to draw biological conclusions. Effective de-listing of endangered subspecies (or species) should be based on an exhaustive analysis of the species' biology, not a cursory look at an infinitesimally small, peculiar, uniparentally-inherited DNA sequence. When consequences are irreversible, accounting for uncertainty is important.

A second investigation regarding *Z. h. preblei, Z. h. campestris,* and other *Z. hudsonius* subspecies (King et al, 2006) presents additional analysis of phylogeographic structure within *Z. hudsonius.* This investigation provides conflicting findings compared to earlier work (Ramey et al, 2005) and demonstrates that *Z. h. preblei* and *Z. h. campestris* can be genetically differentiated from each other. King et al, 2006, presented strong evidence that these two subspecies are genetically distinct. We also believe that the King study is a more extensive, rigorous investigation and is a more reliable source of genetic/scientific foundation for *Zapus hudsonius*, especially when considering protection under ESA. Despite these findings, it is obvious that there is at least great uncertainty regarding the genetics of these two taxa, and we urge the U.S. Fish and Wildlife Service to exercise great caution when considering delisting *Z. h. preblei.* Ramey et al (2005) and King et al (2006) represent the only investigations in this matter and are in wide disagreement concerning subspeciation. It would seem prudent in the sake of species/biodiversity conservation, that a decision should err on the side of conservatism and protect threatened taxa.

Ecology and Status

Z. h. preblei is restricted to riparian and marshy areas in the semi-arid environment of the Colorado Front Range and southeastern Wyoming. We suspect that *Z. h. campestris* is as well, however much less is known about the ecology of this more northern subspecies relative to that of *Z. h. preblei.* Dense vegetative cover is apparently necessary for the maintenance of populations of *Z. h. p.* (Clark and Stromberg 1987, Bakeman 1997). *Z. h. preblei* is known to wander, shifting nightly or seasonally in response to drying conditions or abundance of food sources (Clark and Stromberg 1987, Fitzgerald et. al. 1994, Shenk and Sivert 1999). *Z. h. preblei* are true hibernators spending up to seven months underground in torpor living on fat stores (Meaney et al. 2003). Upland areas adjacent to the riparian linear corridors are also used for hibernacula, cover and food. In order to enter hibernation, individuals need substantial food sources to put on fat stores. This illustrates how *Z. h. preblei* must range widely in a contiguous habitat corridor in order to thrive. Putting on fat stores depends on ready supplies of food at the proper time of year. If habitats are fragmented, individuals may not be able to move to new food sources at critical times of the year or may need to move across areas of poor cover exposing them to predation or other harsh environmental conditions. Due to their habitat requirements and natural history, *Z. h. preblei* requires contiguous riparian corridors with ample vegetative cover.

State natural heritage programs in the U.S. track the status of wildlife and wild plant species, are each state's primary comprehensive biodiversity data center, and conduct studies to document the status of mammal species. In Colorado and Wyoming, *Z.h. preblei* is listed as "critically imperiled due to rarity...or vulnerability to extinction" (CNHP 2005 and Keinath, et al. 2003). *Z. h. campestris* is considered a species of special concern and is listed as "regionally rare and critically imperiled" in Wyoming due to rarity (Keinath, et al. 2003) or as "very rare or only locally abundant" in, Montana (MNHP 2004) and South Dakota (SDNHP 2005) where this rodent is known to occur. These databases illustrate the rarity of this taxon and the concern for its conservation.

Biogeography is the science that describes the pattern of species distribution at regional and continent-wide scales (McDonald 2003) and can help us understand the role of "physiographic barriers" on the high plains of Wyoming and Colorado. These barriers prevent movement and apparently limit gene flow between *Z. h. preblei* and *Z. h. campestris*, and within populations of *Z. h. preblei*. These natural and man-made barriers include:

1. A Wyoming geographic barrier creates a break between populations of *Z. h. preblei* and *Z. h. campestris* as illustrated in *Mammals in Wyoming* (Clark and Stromberg 1987). Specifically, this region, north of the North Platte River watershed is the dry uplands of east-central Wyoming in the vicinity of Thunder Basin National Grassland, including basins of the Niobrara River, South Fork Powder River, and low-elevation portions of the Cheyenne River (Douglas A. Keinath, Wyoming Natural Diversity Database, personal communication). Although not thoroughly sampled, trapping efforts in this region to date have not produced any *Zapus* captures (Gary Beauvais, Wyoming Natural Diversity Database, personal communication). We believe this area is a physiographic barrier to movement between *Z. h. preblei* and *Z. h. campestris* in Wyoming. Other species including butterflies (DeChaine and Martin 2004), beetles (Noonan 2001) and plants are known to be limited by this physiographic barrier dividing the North Platte River from watersheds to the north.

2. The Denver Metro Area presents a barrier to movement by *Z. h. preblei* populations in the northern to southern portions of the South Platte River watershed. The U.S. Fish and Wildlife Service has granted a block exclusion area for the Denver area (USFWS 2004), which lies in the middle of this watershed.

Considering these barriers to movement, between subspecies *Z. h. preblei* and *Z. h. campestris* in Wyoming and between populations of *Z. h. preblei* in Colorado, supports the idea that genetic separation of these groups within this taxon is a highly likely phenomenon. Given current climatic and geographic conditions, these separations will likely exist for many generations creating the potential for increased genetic divergence among these groups. Recent genetic analysis of these taxa supports the idea that barriers do exist (King et al., 2006).

Population Vulnerability

There are few long-term population data to indicate whether *Z. h. preblei* is declining or increasing within its known range. *Z. hudsonius* population data were collected from several sites within Colorado in the late 1990s to assess population status. Using comparable field methods and data analysis techniques, it was determined that the mean linear density of *Zapus* from several Colorado sites was 32 animals km^{-1} of stream (White and Shenk 2000). Recent, longer term data at some sites indicates that the 32 animals km^{-1} reported in the late 1990's, may overestimate mean relative abundance at many sites. We do not know of any comparable studies within the range of *Z. hudsonius preblei* in Wyoming.

Most of these study areas were surveyed for two years, but there were three areas in particular for which longer term population data were collected: South Boulder Creek (4 years of data, Boulder County), East Plum Creek (7 years of data, Douglas County) and Dirty Woman Creek (7 years of data, El Paso County).

Data from 1997-2000 along South Boulder Creek show a high degree of population variation between trapping sessions and sites (7 sites, 2 trapping sessions per year, 4 years). For example, linear population estimates ranged from 0 to 105 Preble's km^{-1}At

discernable trends over the 4 years, and it was suggested that a period of 10 or more years may be necessary to assess trends in this subspecies.

Data from East Plum and Dirty Woman Creeks also show a high degree of population variability between years. Population estimates peaked in the late 1990s at both sites, and crashed in 2002, a drought year. One site at the East Plum Creek study area had a 1999 linear density estimate of > 200 Preble's km^{-1} of stream (the highest recorded density of any site to date), but declined to 0 Preble's km^{-1} of stream in 2002. Recent data at the Dirty Woman Creek site show a statistically significant 56% reduction in *Z. h. preblei* relative abundance following three years of highway construction (Bakeman; personal communication). These data indicate that *Z. h. preblei* populations are sensitive to both natural stochastic processes and anthropogenic disturbances.

The above illustrate the following points: 1) long-term data on *Z. h. preblei* populations are generally lacking for much of the known range, 2) at sites where population data have been collected for more than two years, it appears that populations of *Z. h. preblei* are highly variable and can be reduced to dangerously low levels at local scales, and 3) the mechanisms that cause this variability are unknown.

Threats

Within the South Platte and Arkansas watersheds in Colorado, *Z. h. preblei* is known from some of the most rapidly developing areas in the United States. Douglas County was the fastest growing county in the United States from 1990 to 1999, with a population increase of 160% (U.S. Census Bureau 2005). El Paso County grew by 102,980 people during this same time frame (25.9% increase), and is projected to have an additional 200,000 new residents by 2025. One only needs to read the local newspaper to know that northern portions of the South Platte River watershed in Larimer and Weld counties are enjoying unprecedented growth in recent years. Both upland and riparian habitat of *Z. h. preblei* in these watersheds has been directly and indirectly affected by these changes, with habitat fragmentation and loss of riparian habitat and function some of the more serious consequences.

Increased development and associated hard surfaces affects stream hydrology by increasing stream surface flow (and energy). This leads to channel incision and a lowering of the alluvial groundwater table, with negative consequences for riparian vegetation. Many plants and animals are impacted; especially the riparian obligate *Z. h. preblei*.

It is evident that considerable former *Z. h. preblei* habitat has been lost. Ryon (1995) discovered no *Z. hudsonius* at sites where they were captured historically throughout the Front Range Urban Corridor of Colorado. Habitat loss and land conversions were given as likely causes (threats) for why *Z. h. preblei* was no longer present at these sites. Recently, USFWS (2004) has granted "Block Exclusions" for the Denver and Colorado Springs metropolitan areas. These exclusion areas indicate locations where habitat is unlikely to occur in areas that likely had habitat at one time. *Z. h. preblei* habitat has been especially fragmented within the Arkansas watershed in El Paso County. In the late 1990's this was considered the largest population of *Z. h. preblei*, and consisted of mainstem Monument Creek through the U.S. Air Force Academy and 7-11 occupied tributaries. All these tributaries have been fragmented and presently there are 7 small, disconnected populations, vulnerable to extirpation due to their small size and isolation. If delisting occurs, the incentive to carry out important conservation programs that are in progress, such as restoration of movement corridors, will likely fade and eventually fail.

Furthermore, recent trapping data suggest that montane populations of *Z. h. preblei* in the Pike National Forest are very low, possibly a result of habitat fragmentation and loss, especially following the extensive Hayman Fire of 2002. *Zapus* capture rates at the intermediate-elevational zones of the Pike National Forest are consistently low despite enormous trapping efforts. For example, following a three-year study in six drainages of the Pike National Forest, there were only 40 distinct *Zapus* captures and the capture rate specific to *Zapus* was 0.39 percent (Hansen; personal communication). Telemetry and trapping data from this study revealed that montane *Z.h.preblei* never ventured beyond its specialized riparian habitat into wildfire burn areas, flood zones, or developed land regions. Additionally, there was no evidence of *Z.h.preblei* movement between drainages or utilization of the forested upland slopes adjacent to the riparian ecosystem. Similar to meadow jumping mice of the Front Range Urban Corridor, *Z.h.preblei* in the Pike National Forest exhibited its specialized ecological requirements as a riparian obligate, underscoring the importance of the sparse, montane riparian ecosystem to its viability. Delisting of the Preble's mouse would allow federal and state land managers to proceed with forest treatment projects, such as mechanical thinning or prescribed burning, unrestrained by regulations that control activities within the scarce, montane riparian corridors. The specific impacts of these projects on *Z. h. preblei* in montane drainages, are not fully understood, yet likely would subject the already vulnerable mountain populations to additional risk.

Some have indicated that *Z. h. preblei* has a larger distribution now that we have provided funding to trap new areas. A likely quote is "the more we look, the more we find." However, having a better understanding of the distribution of *Z. h. preblei* does not infer that delisting this supbspecies is warranted. The threats that negatively impact habitat have not been removed or sufficiently reduced despite the fact that the distribution of *Z. h. preblei* is different than what we once thought (Schorr 2001). The threats are more widespread and the discoveries of more individuals on the fringes of the distribution range, such as in montane regions, only underline that the taxa is vulnerable. Within the distribution range, habitat has become greatly fragmented and converted to

non-natural uses. The threat of increasing loss and fragmentation of stream habitat, coupled with highly variable *Z. h. preblei* populations and a need for variable habitats in the active and hibernation periods, shows the increased vulnerability of this species for long-term survival. Despite the genetic uncertainties, the *Zapus hudsonius* in the semi-arid west, especially Colorado, are not rebounding but declining and in threat of becoming endangered, in our opinion.

Conclusions

One of the primary reasons for listing *Z. h. preblei* in 1998 was loss of habitat, and this threat has not changed in Colorado. The Preble's Mouse Recovery Team has recommended various population size goals (small, medium, large and combinations thereof) for protection in Colorado counties within the known range. Even if these population goals are met within the designated watersheds (and there has been little progress in this area), the best-case realistic scenario for *Z. h. preblei* recovery within the Colorado Piedmont and the western edge of the Great Plains of Colorado is simply stability; protection of existing populations and habitat. Delisting will result in further continuation of habitat loss and fragmentation, and many of the smaller populations in the Colorado Front Range will be extirpated.

The current body of knowledge for *Z. h. preblei* concerning genetics, ecology and population vulnerability is extremely uncertain. We simply do not know enough to make sound conservation decisions. A basic premise of conservation biology is to be conservative in the face of uncertainty until such time that enough is known to make educated decisions (Pague and Grunau 2000) especially when concerning small populations (Soule and Wilcox 1980). To proceed with a delisting of *Z. h. preblei* based largely upon conflicting genetic data (Ramey et al, 2005 and King et al, 2006) for this taxon does not appear justified.

We consider *Z. h. preblei* to be an extremely vulnerable taxon worthy of continued federal protection. The best available science regarding genetics and abundance does not warrant delisting. Real estate development of the Colorado Front Range Urban Corridor will continue. If federal protection is removed now, we will only find ourselves, as a community, returning to list meadow jumping mice when the habitat and our waterways are further degraded sometime in the future. It is far less expensive to maintain protection now than to try to recover this subspecies from the brink of extinction later.

This letter is respectfully submitted by the following individuals:

Carron Meaney, Ph.D., Meaney & Company, Research Associate, Denver Museum of Nature and Science and Curator Adjoint, University of Colorado Museum

Carron Meaney

Thomas Ryon, M.S., Wildlife Biologist and Certified Ecologist, Ecological Society of America

Thomas R. Ryon

Mark Bakeman, Ph.D., President, Ensight Technical Services Inc.

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Anne K. Ruggles

Additionally, the following individuals support the comments provided above:

David M. Armstrong, Professor, Department of Ecology & Evolutionary Biology, and Environmental STudies Program, University of Colorado-Boulder

Norm Clippinger, PhD., Research Associate, Dept of Ecology and Evolutionary Biology, University of Colorado, Boulder

Dr. David J. Hafner, Chair and Curator, Biosciences Department, New Mexico Museum of Natural History Albuquerque

Craig Hansen, Master of Science in Biology, University of Colorado, Colorado Springs

Cheri Jones, Ph.D., Senior Instructor University of Colorado at Denver and Health Sciences Center

Andrew Martin, Ph.D., Associate Professor, Dept of Ecology and Evolutionary Biology, University of Colorado, Boulder

References

Bakeman, M.E. ed. 1997. Report on habitat findings of the Preble's meadow jumping mouse. Boulder (CO): Report to the U.S. Fish and Wildlife Service and Colorado Division of Wildlife. 91 pp.

Clark, T.W., and M.R. Stromberg, 1987, Mammals in Wyoming. Public Education Series of the Museum of Natural History, University of Kansas 10:1-314.

CNHP 2005. Statewide List of Tracked Mammals. Prepared by the Colorado Natural Heritage Program – Colorado State University, Fort Collins, Colorado.

DeChaine EG, Martin AP (2004) Historic cycles of fragmentation and expansion in *Parnassius smintheus* (Papilionidae) inferred using mitochondrial DNA. Evolution 58: 113-127.

Fitzgerald, J.P., C.A. Meaney, and D.M. Armstrong. 1994. Mammals of Colorado. University Press of Colorado and Denver Museum of Natural History. 467pp.

Keinath, D., B. Heidel and G. P. Beauvais. 2003. Wyoming Plant and Animal Species of Concern. Prepared by the Wyoming Natural Diversity Database - University of Wyoming, Laramie, Wyoming.

King, T.L., J.F. Switzer, C.L. Morrison, M.S. Eackles, C.C. Young, B Lubinski, and P. Cryan. 2006. Comprehensive analysis of molecular phylogeographic structure among the meadow jumping mice (*Zapus hudsonius*) reveals evolutionarily distinct subspecies. U.S. Geological Service. Report submitted to the U.S. Fish and Wildlife Service.

MacDonald, G. M. 2003. Biogeography: Introduction to Space, Time and Life. John Wiley & Sons.

Meaney, C.A., A.K. Ruggles, B. Lubow, N. Clippinger. 2003. Abundance, survival, and hibernation in Preble's meadow jumping mice (*Zapus hudsonius preblei*) in Boulder County, Colorado. The Southwestern Naturalist, 48(4):610-623.

MNHP 2004. Montana Animal Species of Concern. Prepared by the Montana Natural Heritage Program and the Montana Fish Wildlife and Parks. A program of the Natural Resource Information System, Montana State Library. Website: http://nhp.nris.state.mt.us

Noonan GR (2001) Systematics and cladistics of the North American subgenus *Anadaptus* Casey (Genus *Anisodactylus* Dejean) and a geographic information system Analysis of the Biogeography of Included Species. Ann. Entomol. Soc. Am. 94: 301-332.

Pague, C.A. and L. Grunau. 2000. Conservation Planning Handbook for the Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*); with guidance from the Preble's Meadow Jumping Mouse Science Team. Prepared for The Colorado Department of Natural Resources. Colorado Natural Heritage Program. Fort Collins, Colorado.

Ramey, R.R., H Liu, C.W. Epps, L.M. Carpenter, and J.D. Wehausen. 2005. Genetic relatedness of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA and microsatellite DNA: implications for taxonomy and conservation. Animal Conservation (8), 329–346.

Ryon, T.R. 1995. The evaluation of the historic capture sites of the Preble's meadow jumping mouse in Colorado. Masters Thesis, Department of Environmental Science, University of Colorado at Denver.

Schorr, R. A. 2001. Meadow jumping mice (*Zapus hudsonius preblei*) on the U.S. Air Force Academy, El Paso County, Colorado. Colorado Natural Heritage Program unpublished report to the Natural Resources Branch, U.S. Air Force Academy. 53pp.

SDNHP 2005. Rare, Threatened or Endangered Animals Tracked by the South Dakota Natural Heritage Program. Prepared by the South Dakota Natural Heritage Program. Website: http://www.sdgfp.info/wildlife/diversity/index.htm#Heritage

Shenk, T.M. and M.M Severt . 1999. Movement Patterns of Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*) as They Vary across Time and Space. Appendix A in: Wildlife Research Report, Part I (July); Mammals Program. Colorado Division of Wildlife Research Center, Fort Collins, Colorado.

Soulé, M.E . 1980. Thresholds for Survival: Maintaining Fitness and Evolutionary Potential in: *Conservation Biology: An Evolutionary-Ecological Perspective*. Soulé, M.E. and B.A. Wilcox, ed. Sinauer Associates, Inc. Sunderland, Massachusetts.

United State Census Bureau. 2005. American Fact Finder. http://www.census.gov/popest/archives/1990s-CO-99-06/99c6_08.txt. 31 March 2005.

U.S. Fish and Wildlife Service. 2004. Denver Block Exclusion for the Urban Drainage and Flood Control District. Letter to Ron Beane of EROS Resources from Lakewood Field Office, Lakewood, Colorado.

White, G.C. and T.M. Shenk. 2000. Population estimation with radio-marked animals. Proceedings of The Wildlife Society Symposium: Analysis of Radiotelemetry Data (J.J Millspaugh, J.M. Marsluff, and M. R. Fuller, eds.).

RE: DELISTING OF PREBLE'S MEADOW JUMPING MOUSE

It's maddening to me how life gets pushed aside in the name of development. Even the smallest creatures are an essential part of our ecosystem. All life is precious. So please, I'm asking you, do not delist this animal or the Great Plains won't be quite as great. Thank you for your time.

Sincerely,
Tina Comerford
Wheaton, IL

RECEIVED
MAY 22 2006

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May 17, 2006

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Via Facsimile: 303-236-4005
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RE: Comments on Delisting of the Preble's Meadow Jumping Mouse

To Whom it May Concern:

While I have generally been aware of the issues relating to the Preble's Meadow Jumping Mouse ("Preble's mouse") in Colorado and Wyoming since it was designated by the U.S Fish and Wildlife Service ("USFWS") in May, 1998, I did not have a personal stake in its regulation and protection until recently. I offer the following comments as just one example of the impacts, both economic and environmental, that are occurring in Wyoming because of the Preble's mouse "protections". I urge the USFWS to remove the limitations on land use activities within Wyoming by delisting the Preble's mouse.

I own lands located approximately 35 miles northwest of Laramie in Albany County, Wyoming, which are allegedly part of the Preble's mouse "historic range". My goal with any piece of property that I own is to improve it. More specifically, I expend resources to enhance the property's habitat conditions for fish and wildlife purposes.

After I purchased the property, I retained an engineering firm to survey the property to identify potential locations of small reservoirs to provide stockwater and develop habitat for fish and wildlife propagation. The engineering report recommended the construction of two small reservoirs for these purposes. The first reservoir was designed for a capacity of 6.5 acre-feet with a wetland impact of .13 acres. The second reservoir was designed for a capacity of 18.28 acre-feet with a wetland impact of 1.09 acres. The total project wetland impact is less than 1.25 acres.

I decided that I wanted to construct these two reservoir so I filed two applications with the Wyoming State Engineer's Office in July, 2002. I understood there would be some regulatory issues to deal with so we planned to construct the reservoirs in 2004. When we requested a permit from the Army Corps of Engineers ("ACOE") in July, 2002, we were informed that the Preble's mouse was listed as threatened and that my property is located in its historic range. The USFWS was then contacted to find out what was necessary to satisfy the ACOE consultation requirement. We were informed that a survey must be conducted on the

property. When we contacted an environmental engineering firm to conduct the survey, I was informed that the survey costs would be approximately $10,000. This amount does not include the costs of any mitigation activities that would likely have been required by the USFWS. I did not understand why such a survey would be so expensive until I reviewed the overwhelming requirements listed in the "USFWS Interim Survey Guidelines for Preble's Meadow Jumping Mouse, Revised May 19, 1999". The guidelines include strict requirements for survey permitting qualification, numbers of traps to be set, and detailed reporting mandates. It seemed to me that this high-priced experiment was not worthwhile when I learned that no Preble's mouse has ever been discovered in surveys in the area of my property. I became further disillusioned with the requirements and the general goal of Endangered Species Act ("ESA") protections when I learned that the survey did not in fact protect the Preble's mouse, but instead would kill it in the trapping survey. My disbelief and confusion resulted in my decision to not proceed with my habitat improvement project due to the costs required to comply with the Preble's mouse regulations.

I am convinced that the Preble's mouse regulations are preventing habitat improvement projects just like mine in both Wyoming and Colorado. My conviction caused me to review the "Economic Impact Analysis of Critical Habitat Designation for the Preble's Meadow Jumping Mouse" prepared by Industrial Economics, Inc. The study acknowledges that 65% of the lands affected by the Preble's mouse regulations are privately owned and, therefore, will bear the economic brunt of complying with these regulations.

The study concludes that consultation and project modifications involving the Preble's mouse are expected to cost $79 to $183 million over the next ten years, or approximately $8 to $18 million per year. The study also concludes that the critical habitat designation in Wyoming is expected to have a "modest" impact on agricultural land use. It is abundantly clear that we have a different definition of "modest".

What this economic study does not analyze are the opportunity costs associated with potential agricultural development. In my opinion, the confusion, fear and uncertainty regarding the Preble's mouse has had a chilling effect on private landowners' willingness to make habitat improvements even if they don't require a federal permit. For those projects that do involve a federal permit, typically one call to the federal agency that explains the trapping requirements, etc. necessary in the Preble's mouse habitat range doom any project. These "costs" are not discussed, let alone quantified in the economic report.

The economic analysis attempts to offset these enormous economic costs by listing the many "benefits" of protecting Preble's mouse habitat. None of the benefits listed (decreased habitat loss, decreased destruction of riparian habitat, preservation of open space, enhanced recreation, and improved ecosystem health) were accomplished by preventing construction of the reservoirs on my property. I assume that is true in many other instances as well.

The fact remains that the most definitive scientific work on the Preble's mouse was completed by Dr. Ramey. Dr. Ramey has suffered tremendous personal and professional costs for stating his scientific conclusion that runs counter to the goals of environmentalists. Dr. Ramey's work has been peer reviewed and scientifically accepted. Even scientists who disagree with Dr. Ramey admit that the Preble's mouse is 99.5% genetically similar to other strains of mice. The USFWS will always be able to find other scientists to disagree with Dr. Ramey's conclusions. I encourage the USFWS to stop its scientific shopping excursion, accept Dr. Ramey's conclusions and delist the Preble's mouse.

Thank you for your consideration and the opportunity to comment on this proposal.

Sincerely,

Neil A. "Mick" McMurry
Shareholder

ramey@spot.colorado.edu
05/18/2006 11:45 PM

To FW6_PMJM@fws.gov
cc Dale_Hall@fws.gov
bcc
Subject Comments on Preble's meadow jumping mouse

Please consider the attached comments on Preble's meadow jumping mouse for the record.

Attached files:
Response to VEA 5_15_06.pdf
Our response paper to Vignieri et al. that was recently submitted to *Animal Conservation*

Comments on King by Ramey et al.pdf
Our comments on the unpublished report by King et al.

Thank you,

Rob Roy Ramey II, Ph.D.
P.O. Box 386

Nederland, CO 80466

Response to VEA 5_15_06.pdf

Comments on King by Ramey et al.pdf

Should hypothesis testing or selective posthoc interpretation of results guide the allocation of conservation effort: a response to Vignieri *et al.*

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5/15/06

In their response to Ramey *et al.* (2005), Vignieri *et al.* (this issue; hereafter VEA) claim that they are concerned about erroneous application and interpretation of morphometric, genetic, and ecological data. We share this concern, which is why we used a consistent hypothesis-testing approach to test the taxonomic validity of the Preble's meadow jumping mouse (*Z. h. preblei*) as a subspecies and its uniqueness as a distinct population segment. We used critical tests that were set in advance of data collection to avoid subjective posthoc interpretation of results. We also used multiple lines of evidence for our tests of uniqueness to avoid erroneous conclusions (Ramey *et al.* 2005). We do not agree with VEA that four lines of corroborating evidence can be considered to be "narrow in scope".

Contrary to their stated goals, VEA did not accurately portray our goals, methods, results, or conclusions. They selectively cited information and relied on speculation and post hoc interpretation of results to support their claims that *Z. h. preblei* is a distinct subspecies and an "evolutionary distinct mouse." We contend that the approach used by VEA was less than objective, and if widely applied, could result in the misallocation of conservation effort to many non-distinct local populations.

Morphometric Analyses

At the center of this debate is the separation of *Z. h. preblei* as a subspecies by Krutzsch (1954) based on measurements of only 3 skulls and comparisons of only 4 skins – sample sizes that no modern taxonomist would accept. In their attempt to defend this taxonomy, VEA try to discredit all of our morphometric analyses, while ignoring the work of Jones (1981) that found no morphological support for any subspecies of *Z. hudsonius*. VEA state that our analyses suffered from intercorrelated data because 26 of 36 correlations among the 9 skull measurements were significant at $P < 0.001$. Yet, these were the same measurements used by Krutzsch (1954), whose conclusions they attempt to defend. Traditional frequentist statistical tests that emphasize P values have come under strong criticism (Cherry 1998, Johnson 1999; Anderson, Burnham, and Thompson 2000). Indeed, the P values that VEA cite for correlations in our data reflect the large cumulative sample size we used, rather than statistically important levels of correlation among variables used in multivariate analyses of shape variation.

Krutzch's sample sizes precluded meaningful statistical tests, and he used none; yet, VEA concluded that his finding of a smaller interorbital breadth in *Z. h. preblei* was a "definitive finding". VEA claim that interobital breadth was the only one of the morphometric variables we measured that Krutzsch (1954) found to distinguish *Z. h. preblei* and that our finding of a difference for that character confirmed Krutzsch's (1954) conclusion. What Krutzsch (1954) actually stated was that *Z. h. preblei* was smaller than *Z. h. campestris* in most of the nine skull dimensions measured, a hypothesis that our data clearly refuted. Such univariate tests that VEA appear to espouse were replaced decades ago in morphometric analyses by multivariate analyses of shape variation (Reyment Blackith, & Campbell 1984), which was the approach we used.

VEA criticize us for ignoring unquantified characters that Krutzsch (1954) included as the basis of his taxonomy, describing these as "shape differences noted by a trained morpho-taxonomist". They fail to realize that this "trained morpho-taxonomist" (Krutzsch) does not accept his taxonomy and has publicly stated that our research "*clearly invalidates Z. h. preblei and demonstrates its relationship to Z. h. campestris.*" He went on to state: "*Perhaps most significant is the model you provide to unequivocally establish the uniqueness of an organism and its relationships before declaring it in danger of extinction. Such an analytical approach would prevent implementation of a process to support an agenda or a point of view. I can think of other listed endangered species that could have benefited for a prior, detailed, scientific appraisal*" (P. Krutzsch in e-mail to R.R. Ramey, entered into the U. S. Congressional Record on 28 April 2004).

Ecological Analysis

Contrary to VEA's claims, we did not deny that *Z. h. preblei* seems to be currently isolated. What we questioned was *how long* this isolation has existed. Nor did we "present nothing" that could be interpreted as a test of ecological exchangeability. We cited the original morphological research of Krutzsch (1954) and Jones (1981) as well as the literature reviews Whitaker (1972, 1999), Clark & Stromberg (1987) and Cryan (2004) in support our claim that no adaptive differences have been described between *Z. h. preblei* and other subspecies. While it is possible that some critical adaptive difference had been "missed" in the 106 years of study, starting with Preble (1899), none seem to have been noticed.

VEA make the assertion that "*the potential for ecological differentiation among these populations (putative subspecies of Z. hudsonius) is high.*" However, the evidence and rationale they provide is speculative. VEA base their claims on Kuchler's (1964) Potential Natural Vegetation (PNV) classifications. PNV classifications are based on hypothetical "climax" vegetation that could potentially occupy a site without disturbance or climatic change (Stephan 1998). PNVs are not mutually exclusive categories. For example, each of the PNVs cited in VEA has overlap in plant species. PNV classifications are qualitative, generalized descriptions of vegetation communities that do not take into account the mosaic nature of natural landscapes, including successional stages, nor do they accurately characterize moist riparian habitat occupied by *Z. hudsonius* in the Great Plains. VEA ignore the fact that *Z. hudsonius* is a generalist species in its food habits (eating seeds, insects, fruit, and fungi) and habitat preferences (Quimby 1951, Jones 1981) making adaptation to specific forage species less likely. VEA's assertion that the potential for ecological differentiation is high is therefore questionable. Most importantly, speculation is an inappropriate basis for definitions of subspecies or lower levels of population distinction (Ball & Avise 1992; Crandall *et al.* 2000; Cronin, *in press*); yet VEA declared *Z. h. preblei* a "habitat-specific subspecies group."

Molecular Genetic Analyses

VEA have made a case on the small value of the unscaled migration rates (m) derived from our analyses of mtDNA variation, mistakenly suggesting these rates reflect the number of migrants per generation. In fact, the scaled migration rates ($N_e m$) reflect a theoretical number of migrants per generation of 0.09-0.87 among putative subspecies. While this value is low and suggests the possibility of continuing divergence due to genetic drift, we consider the relative ranking of gene flow rates between putative subspecies as more informative. This analysis suggests that *Z. h. preblei* and *Z. h. campestris* have recently experienced gene flow at higher levels than any other comparison except *Z. h. campestris* and *Z. h. intermedius*.

VEA inaccurately report that reciprocal monophyly was the sole criterion we used for accepting divergence among subspecies. VEA seek to explain away the shared haplotypes among subspecies by labeling them as "contaminant" haplotypes, rather then acknowledging that shared variation is a common biological phenomenon. They attribute this "contamination" to incomplete lineage sorting. Their Table 1 shows that 22.6% of *Z. h. campestris* mtDNA sequences were *Z. h. preblei* haplotypes. This is hardly incomplete lineage sorting.

VEA seek to invoke selective posthoc interpretations to explain away our microsatellite results. They equate statistical significance (in F_{ST}) with biological significance and selectively cite other mammal subspecies comparisons in support of their claim of "strong differentiation" of *Z. h. preblei*. VEA incorrectly report that "95% of the northern population of *Z. h. preblei*" was assigned. What we did find was that 94% of the southern population could be assigned (Table 6, Ramey *et al.* 2005) but we did not use any cut-off value for confidence of assignment (q). Therefore, some of these assignments were only slightly better than coin flips. VEA contradict themselves in stating that we "employed too few loci" while also concluding that our microsatellite results add "further strong support of differentiation" of *Z. h. preblei*.

Z. h. preblei and the US-Endangered Species Act

VEA suggest a double standard in evaluating evidence used in ESA listings. They state that Ramey *et al.* (2005) "should most certainly not be presented as an adequate basis for the making of taxonomic decisions regarding a (US-ESA) listed taxon". Yet they ignore the fact that *Z. h. preblei* was US-ESA listed based on far fewer data -- Krutzsch's (1954) study of just a few specimens and an unpublished qualitative mtDNA study for which that data were never made public (Riggs *et al.* 1997).

VEA raise some important questions with regards to subspecies and populations relative to the ESA. How should conservation effort be allocated relative to (1) hypothesized adaptive uniqueness; (2) geographic isolation of recent origin; and (3) populations showing minor differentiation at few neutral loci that may be due to recent anthropogenic population bottlenecks?

We agree with VEA that it is impossible to predict future patterns of speciation. However, the US-ESA is not a biodiversity law that mandates the protection of all potential pathways to speciation (e.g. weakly differentiated populations or hypothetical evolutionary trajectories). VEA's suggestion that the ESA should protect all potential speciation pathways is impractical, logically inconsistent, and not a view supported by the courts. It is impractical because there is great uncertainty in predicting potential speciation pathways. It is logically inconsistent because the evolutionary potential for some species can only be realized through the extinction of other species (e.g. in cases where one species is limited by another), leading to conflicting listing and recovery goals Lastly, VEA's approach is in conflict with a recent U. S. Ninth Circuit Court ruling that while *"the USFWS can draw conclusions based on less than conclusive evidence, ...it cannot base its conclusions on no evidence"* (National Association of Homebuilders vs. Norton, No. CIV-00-0903-PHX, 2001). In other words, US-ESA decisions cannot be based on speculation or hypothetical scenarios alone.

In listing *Z. h. preblei* as "threatened", the USFWS concluded that there was a loss of populations over a significant portion of its range (USFWS 1998). Post-listing surveys have shown this conclusion to be erroneous. Historically (pre-1980), the range of *Z. h. preblei* was thought to be restricted to fourteen 8th order hydrologic units along the eastern edge of the Rocky Mountains in Colorado and Wyoming (State of Wyoming 2003; data from Wyoming Natural Diversity Database and Colorado Natural Heritage Program), of which 9 were thought to be occupied at the time of listing based on minimal survey efforts (USFWS 1998). This rodent is now known to occur in all historically occupied hydrologic units in both Colorado and Wyoming. In addition, it has been captured in three hydrologic units north and east of its presumed historic range: the Upper Laramie Hydrologic Unit in Wyoming as well as the Kiowa and Chico Hydrologic Units in Colorado (State of Wyoming 2003 - see Table 4-5). While development and habitat alteration have certainly caused some local extirpations, the number of occupied locations within these hydrologic units has increased over fourfold with greater survey effort, to over 126. Consequently, it appears that data on taxonomic uniqueness and geographical distribution used for ESA listing were both questionable. Yet, VEA propose to maintain the status quo of *Z. h. preblei* under the ESA. This raises fundamental questions regarding the allocation of conservation effort.

The U.S. Government Accountability Office recently reported that the time and costs that are required to recover US-ESA listed species, subspecies, and distinct vertebrate populations are largely unknown (U.S. Government Accountability Office 2006). With costs and duration of most US-ESA listings unknown, it would seem that prioritization in the allocation of conservation effort would become imperative. However, this has not been the case. Although a prioritization scheme was established in the 1982 amendments to the US-ESA it was based on taxonomic uniqueness, and it has subsequently been found that there is no correlation between priority rank and conservation expenditure (Restani and Marzulluff 2001, 2002). In other words, expenditures on local populations of otherwise common species (like *Z. h. preblei*) often exceed the expenditures for full species that are at greater risk of extinction. For example, in a ranking of US-ESA

expenditures in 2004, *Z. h. preblei* ranked 125 out of 1260 listed taxa (USFWS 2006). That put spending for *Z. h. preblei* well above that for blue whales - an endangered species (rank 391) and only slightly behind the California Condor -- an endangered monotypic genus (ranked 119).

In the case of *Z. h. preblei*, the only verifiable figures on the cost for the 23,632 ha critical habitat designation was conservatively estimated by the USFWS at $79 to $183 million from 2005-2015 (USFWS 2003). Virtually all of these funds will be spent on consultations rather than more permanent protection, such as land purchases or conservation easements. The development of long-term regional habitat conservation plans accounts for less than 4% of the expenditures. The estimate does not include costs incurred between the time of the listing and the designation of Critical Habitat from 1998 to 2003. It is conceivable that the total allocation of conservation effort for this population could exceed half a billion dollars within the next 20 years.

The U.S. may be unique in its ability to allocate such resources to non-distinct but presumably threatened populations of common species. However, it is clear that this conservation approach comes at the expense of many full species that are far more endangered. With many full species endangered worldwide, and limited resources to save them, many nations may not find the US-ESA model to be a desirable or sustainable approach to conservation.

Literature Cited:

Anderson, D. R., Burnham, K. P. & Thompson, W. L. (2000). Null hypothesis testing: problems, prevalence, and an alternative. J. Wild. Man. **64**:912-923.

Cherry, S. (1998) Statistical tests in publications of The Wildlife Society. *Wildl. Soc, Bull.* **26**:947-953.

Clark, T. W. & Stromberg, M. R. (1987) *Mammals in Wyoming*. Lawrence: University of Kansas Museum.

Cronin, M. A. (in press) Commentary: A proposal to eliminate redundant terminology for intra-species groups. *Wildl. Soc. Bull.*

Cryan, P. M. (2004) Synthesis of existing information on meadow jumping mice (*Zapus hudsonius*) in the northern Great Plains. An information review compiled for the U.S. Fish and Wildlife Service, Region 6, Denver, Colorado. USGS Fort Collins Science Center, 2150 Centre Ave., Building C, Fort Collins, CO 80526. 27pp.

U. S. Government Accountability Office (2006) Endangered Species: Time and Costs Required to Recover Species Are Largely Unknown. GAO-06-463R. Washington, D.C.

Johnson, D. H. (1999) The insignificance of statistical significance testing. J. Wildlife Management **63**:763-772.

Jones, G. S. (1981) The systematics and biology of the genus *Zapus* (Mammalia, Rodentia, Zapodidae) unpublished Ph.D. dissertation. Indiana State University, Terre Haute.

Krutzsch, P. H. (1954) North American jumping mice (genus *Zapus*). *University of Kansas Publications, Museum of Natural History* **4**: 349-472.

Küchler, A.W. 1964. Potential natural vegetation of the conterminous United States: a map and manual. American Geographical Society Special Publ. 36. Princeton Polychrome Press, Princeton, NJ. 116 pp.

Preble, E. A. (1899) Revision of the jumping mice of the genus *Zapus*. *North American Fauna* **15**: 1-41.

Quimby, D. C. (1951) The life history and ecology of the jumping mouse, *Zapus hudsonius*. *Ecological Monographs* **21**: 61-95.

Ramey, R.R., Liu, H. P. Epps, C. W. Carpenter, L. & Wehausen, J. D. (2005) Genetic relatedness of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA, and microsatellite DNA: implications for taxonomy and conservation. *Animal Conserv.* **8**:329-346.

Restani, M. & Marzluff, J. M. (2001) Avian conservation under the Endangered Species Act: expenditures versus recovery priorities. *Conserv. Bio.* **15**:1292-1299.

Restani, M. & Marzluff, J. M. (2002) Funding extinction? Biological needs and political realities in the allocation of resources to endangered species recovery. *Bioscience* **52**:169-177.

Reyment, R. A., Blackith, R. E. & Campbell, N. A. (1984) *Multivariate Morphometrics*. Orlando: Academic Press.

Riggs, L. A., Dempcy, J. M., & Orrego, C. (1997). Evaluating distinctness and evolutionary significance of Preble's meadow jumping mouse: Phylogeography of mitochondrial DNA non-coding region variation. Final Report submitted to Colorado Division of Wildlife. 13 pp. + appendices.

State of Wyoming 2003 - Petition to remove Preble's meadow jumping mouse (*Zapus hudsonius preblei*) from the List of Endangered and Threatened Wildlife. State of Wyoming, Office of the Governor, Cheyenne, WY.

U.S. Fish & Wildlife Service (2003) Final environmental assessment designation of critical habitat for Preble's meadow jumping mouse. Appendix 10.5 Addendum to economic analysis of critical habitat designation for the Preble's meadow jumping mouse. Ecological Services Field Office, Cheyene, Wyoming.

U.S. Fish & Wildlife Service (2006) Federal and state endangered and threatened Species Expenditures: Fiscal Year 2004. U.S. Fish & Wildlife Service Endangered Species Program, Washington, D.C.

Vignieri , S. N., Hallerman, E. M., Bergstrom, B. J., Hafner, D. J., Martin, A. P., Devers, P., Grobler, & P., Hitt, N. (2006) Mistaken view of taxonomic validity undermines conservation of an evolutionarily distinct mouse: a response to Ramey *et al. Animal Conserv.* (this issue).

Whitaker, J. O. (1972) *Zapus hudsonius. Mamm. Species* **11**: 1-7.

Whitaker, J. O. (1999) Meadow jumping mouse (*Zapus hudsonius*). In *The Smithsonian book of North American Mammals:* Wilson, D. E. & Ruff, S. (Eds.). Washington D.C.: Smithsonian Institution Press.

Zerbe, S. (1998) Potential natural vegetation: validity and applicability in landscape planning and nature conservation. *Appl. Vegetation Sci.***1**:165-172.

Is the Preble's meadow jumping mouse an evolutionarily distinct subspecies? Comments on the report by King et al. (2006)

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Introduction

King et al. (2006, hereafter King et al.) produced a second independent set of genetic data on the prebles jumping mouse (*Z. h. preblei*) and their analyses thereof led to the opposite conclusions of Ramey et al. (2005) regarding the uniqueness of that subspecies. King et al. argue that their different conclusions result from sampling design, tissues used (museum specimens versus fresh ear punches), amount of molecular genetic data used (longer and more mtDNA sequences, more microsatellite loci), and analytical methods used, and criteria for uniqueness). We take issue with their interpretation of differences between the studies. Their portrayal of our work was inaccurate and the difference between conclusions is largely a function of basic conceptual differences. While the King et al. sampling represents a notable effort, it falls short in both sampling design and strength of inference that they attempt to present so forcefully.

Morphometric Considerations

King et al. ignored several key sources of information on morphological variation and adaptive differences that did not support their conclusions. These included a literature review conducted by Cryan, one of the coauthors of King et al. (Cryan 2004), who could not find evidence of adaptive differences among putative subspecies, or ecological differentiation that would lead to adaptive differences. Similarly, King et al. ignored the results of Jones (1981) who had found no support for any subspecies of *Z. hudsonius*, despite measuring over 9,000 specimens of *Zapus*.

We also retested the original morphological basis of Krutzsch's (1954) description of *Z. h. preblei* and found no support for his results. That alone would be sufficient basis to reject the taxonomic separation of *Z. h. preblei*. Rather than acknowledging that finding, King et al. dismissed the use of morphology in general because it might not reflect genetic differences, an argument they attempted to support by selective use of references. However, by their argument, these mice should never have been listed under the ESA, a listing that King et al. now strive to defend.

Conceptual Basis and Thresholds for Uniqueness

We based our analyses on a definition of subspecies provided by Ball and Avise (1992) to avoid the long history of taxonomic subspecies decisions having no definitional basis. Ball and Avise (1992) proposed that subspecies represent major subdivisions in the gene pool diversity. By that definition, subspecies are similar to evolutionary significant units (ESUs) as discussed by Moritz (1994a) in requiring deeper historic phylogenetic separation – an important criterion of Crandall et al. (2000). Our uniqueness criteria were set in advance in an attempt to focus on the need for subspecies to represent virtual discontinuities in the gene pool diversity.

King et al. cite Avise and Ball (1990) and Moritz (1994b) as their basis for subspecies and state that they tested the null hypothesis that purported subspecies of *Zapus husdonius* comprise a single homogeneous unit. They claim to accept as evidence of subspecific distinctiveness the conditions previously defined by Avise and Ball (1990) and Moritz (1994b) "as significant phylogeographic separation of mtDNA alleles between subspecies (or populations) combined with congruent phylogeograhic structure for nuclear loci". By their analyses, the definition of subspecies they use differs greatly from ours and appears to be equivalent to what Moritz (1994a) defined as management units. Their different conclusions are substantially a function of these fundamental conceptual differences

In their criticisms of Ramey et al. (2005), King et al. did not acknowledge the ways in which the molecular results of Ramey et al. (2005) were similar to theirs. These include: 1) shallow levels of evolutionary divergence found among putative subspecies for mtDNA and microsatellites; 2) support in mtDNA analyses for a *Z. h. pallidus/luteus* clade and a *Z. h. preblei/campestris/intermedius* clade; 3) putative subspecies were not reciprocally monophyletic or even close to being so; and 4) few unique alleles found in *Z. h. preblei* despite a larger sample size for this putative subspecies. These similarities are important because of their bearing on how different conceptual approaches to subspecies affected differences in conclusions.

Statistical Significance Versus Biological Significance.

In their null hypothesis testing of genetic homogeneity, King et al. equate statistical significance with biological significance – an analytical approach that deviates from ours. It is known that with a large enough sample size it is possible to find statistical significance in almost any comparison, especially when intervening variation is ignored. As pointed out by Hedrick (2001): "*the statistical power for determining differentiation between groups is closely related to the number of independent alleles, so, that for even for a few highly variable microsatellite loci, there can be high statistical power. When there is such high statistical power, extremely small molecular genetic differences between groups become statistically significant.*"

King et al. screened a large numbers of individuals from few populations for a large number of microsatellites (27 total). Although King et al. found a high level of statistical significance in their comparisons (similar to our results), the degree of differentiation among *Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius* were the lowest of any of the pairwise comparisons for mtDNA (King et al. Tables 8 and 9) and microsatellites (King et al. Table 6). This low degree of differentiation is illustrated by the fact that only 4 unique alleles were reported in *Z. h. preblei* (out of 279 total), the lowest number of unique alleles for any subspecies sampled. It appears that King et al. are reporting statistical significance in tests that are of little biological relevance.

King et al. reported high levels of correct assignment to subspecies using the program STRUCTURE. These authors attribute this to *Z. h. preblei* having "considerable evolutionary differentiation" from other subspecies and to shortcomings of the Ramey et al. (2005) study. However, King et al. failed to acknowledge that this high level of correct assignment could be an artifact of sampling design (as discussed below). Moreover, the higher levels of correct assignment also are very likely to result from the larger number of microsatellite loci surveyed. King et al.'s findings raise a valid critique of both studies - use of assignment tests such as STRUCTURE may not be an appropriate tool for evaluating taxonomic separation, because of the sensitivity of these tests to the number of loci employed.

The probability of correct assignment of individuals to populations can increase substantially with the number of microsatellite loci used. For example, in a study that examined the distribution of variation within and among human populations, Rosenburg et al. (2005) found that increasing the number of microsatellite loci increased the statistical significance of assigning individual humans to geographic populations. These authors, who employed 993 microsatellite loci in their study, reported that: "*human genetic diversity consists not only of clines, but also of clusters, which STRUCTURE observes to be repeatable and robust.*" In light of these observations it is worth asking: If a high level of correct assignment can be detected using microsatellite loci, should populations automatically be considered as subspecies or distinct population segments (DPSs)? If King et al.'s approach were applied to human populations, how many subspecies of *Homo sapiens* would King et al. recognize? Future efforts employing these types of analyses may need to establish threshold assignment probabilities for a set

number of loci with a given amount of variation per locus to allow comparability between studies.

Sampling Questions

Sampling Distribution

King et al. sampled many individuals from few local subpopulations (within a 6km radius) within each putative subspecies, whereas Ramey et al. (2005) sampled many populations, but few individuals per population, across the range of each putative subspecies. An ideal study design would incorporate both approaches, thereby sampling many individuals across the range of each putative subspecies. However, this is not often practical because of logistical and funding constraints. Given the choice, which strategy provides the most objective test of subspecies uniqueness?

King et al. claim that their sampling strategy allows more appropriate statistical testing, but they do not provide any supporting evidence. Instead, the sampling approach of King et al. has created artificial gaps in the distribution of genetic variation, making it less appropriate for subspecies comparisons. Most importantly, their sampling approach ignores the variation found across the range of the putative subspecies (or hypothetical DPSs), especially near zones of current or recent contact.

It is notable that King et al. did not make use of available specimens from southeastern Wyoming. These are the areas where shared variation among putative subspecies is most likely to be found (because of current or recent genetic exchange between *Z. h. preblei* and *Z. h. campestris*). Because King et al. sampled only one subpopulation of *Z. h. luteus* and two subpopulations each of *Z. h. campestris*, *Z. h. intermedius*, and *Z. h. pallidus*, artificial gaps have been inserted between all of the subspecies. Therefore, the sampling strategy of King et al. predisposed the results to exaggeration of genetic distances among putative subspecies. This same effect was recently reported by Rosenburg et al. (2005) in comparisons of microsatellite data from human populations.

The sampling scheme of King et al. also created an artificial gap within the range of *Z. h. preblei*. This resulted in the recognition of two potentially listable DPSs under the ESA. As noted in Ramey et al. (2005) the Denver metropolitan area creates a manmade gap in the range of *Z. h. preblei*. King et al. artificially increased the size of that gap (by ~70km) by excluding a large number of samples from Boulder County, Colorado. Furthermore, by not taking into account that growth of the Denver Metropolitan could affect the distribution of genetic variation of mice in this area (e.g. local extirpation and bottlenecks), it appears that the results of King et al. could lead to the identification of DPSs solely based on manmade separation of ranges and recent anthropogenetic changes to the environment.

Despite its limitations in inferring degree of within-population variation, the sampling scheme used by Ramey et al. is (2005) is supported both theoretically and empirically by

other authors for the broader scale comparisons across species (Lynch and Crease 1986; Presa et al. 2002; Rosenburg et al. 2005).

Sources of Material

King criticized Ramey et al. (2005) for using museum specimens, claiming a wide variety of problems associated with such tissue, based mostly on literature for ancient DNA samples, not museum specimens collected within the past 45 years. In truth, Ramey et al. (2005) used a mixture of museum specimens and frozen tissue. However, the specimens used by King et al. are subject to the same issues they raise regarding museum specimens.

Ear punch samples were the primary material used by King et al. and were collected without measures to prevent cross-contamination of specimens. Ear punch samples are small (~1mm in diameter) and collected with a hand-held ear punch tool. Cross-contamination occurs when the DNA of one or more samples is mixed. When the polymerase chain reaction (PCR) is used to amplify copies of DNA from the sample, contaminated DNA is amplified as well, potentially leading to genotyping errors and erroneous results. Because mouse ear punches are minute in size and contain small amounts of DNA these small samples are susceptible to the effects of cross-contamination. As a result, stringent contamination control procedures are needed for sample collection and handling (see Ramey et al. 2000 for procedures used for extracting DNA from individual scabies mites). In the case of King et al., flaming or bleaching of the ear punch tool and forceps would have prevented the carry over of blood or tissue remnants to other ear punch samples. Also, a new pair of laboratory gloves should have been worn when obtaining each specimen to prevent cross-contamination of ear punches as well as the collection tubes, forceps, and sources of ethanol used to fill collection tubes.

Cross contamination in all of the ear punch samples in King et al. is a strong possibility because *the same ear punch tool was used repeatedly to sample multiple mice in all of the ear punch collections.* The samples collected by Cryan and Ellison (2005) were not collected with any of the cross-contamination control procedures discussed above. The ear punch samples collected in Colorado by Schoor and Shenk were only wiped with alcohol (R. Taylor, pers. comm.; Riggs et al. 1997), which cannot be expected to remove contaminating DNA from the inside of the punch tube or reliably remove all contaminating DNA from the outside of the tube. In all cases, there is no documentation that laboratory gloves were ever worn.

Cross-contamination or carryover of genetic material between ear punches is analogous to the spread of infectious diseases through the reuse of contaminated needles. A small amount of contaminating material is all that is needed to produce problems, which is why experienced geneticists go to great lengths to avoid cross-contamination in the collection and handling of specimens.

A difference between King et al. and Ramey et al. (2005) is that the latter relied on vouchered museum specimens. These are accessioned into the collection of an accredited museum with the skin, skull, and detailed collections information to accompany each sample. This means that the specimens used are publicly available and additional follow-up work can be conducted on any sample (e.g. additional genetic analyses, morphometric, and pelage comparisons) whereas the same cannot be said of the ear punch specimens that were used in King et al.

Historic samples, such as vouchered museum specimens, can help the understanding of patterns of genetic variation that occurred in natural populations prior to human induced bottlenecks and local extirpations. If only recently collected samples are used, as advocated by King et al., we limit our ability to investigate the extent to which current patterns of variation are due to natural or recent anthropogenic processes. Ramey et al. (2005) we suggested that "*criteria for genetic uniqueness need to adequately identify natural discontinuities in gene pool variation and distinguish these from recent (e.g. last 100 years) differences that may be due to genetic drift or human-induced bottlenecks or isolation (Hedrick et al. 2001; Brown et al. 2004).*" The use of museum specimens is a part of such an investigative strategy. In contrast, King et al. propose: "*any new study of this type should focus on newly trapped individuals...rather than museum skins.*"

King et al. assert that the shared mtDNA haplotypes found by Ramey et al. (2005) between *Z. h. preblei* and *Z. h. campestris* must have been the result of contamination of museum specimens. Their support for this assertion comes from the claim that they collected a large number of samples from the same locations as the specimens used by Ramey et al. (2005) but did not find any shared haplotypes between *Z. h. campestris* and *Z. h. preblei*. While the potential contamination of museum specimens is a point well taken, there are several reasons to take issue with King et al.'s assertion. They did not consider alternative explanations, did not accurately report the basis of their assertion, and they ignored the fact that Ramey et al. (2005) employed strict contamination control procedures to minimize the chances of cross-contamination or PCR carryover occurring (see Ramey et al. 2000). Additionally, at least two negative controls were used per nested PCR amplification and these did not contain detectable PCR-amplified DNA.

King et al. did not consider the alternative explanations. The first is that the shared mtDNA haplotypes that we observed were actual shared variation rather than contamination. Second, King et al. might not have found these same haplotypes because they have been lost from *Z. h. campestris* due to genetic drift, extirpation and recolonization, or a selective sweep. A similar shift of mtDNA haplotypes, from common to rare, has been previously reported in a study comparing contemporary specimens with museum specimens of *Peromyscus* that were collected one hundred years apart (Pergams et al. 2003). Third, King et al. incorrectly reported that they sampled individuals from the same locations as our museum specimens, but they relied on samples collected over very short distances, typically less than 5km (Cryan and Ellison 2005). At this scale, mice can be expected to be very closely related to each other and not representative of the variation in the population as a whole. Thus they may have sampled different populations of *Z. h. campestris* than Ramey et al. (2005). Fourth, this could be

the result of previously undetected mtDNA heteroplasmy (although we did not see other evidence of this in our study).

Standardization Between Studies

King et al. misrepresented that they had not obtained necessary samples from the Denver Museum of Nature and Science that could have been used to standardize microsatellite alleles between the two studies. It appears that King had obtained *all* of the samples used in Ramey et al. (2005) but either did not run these specimens or did not release the results.

Analysis of Molecular Variance

We concur with King et al. that the AMOVA criterion that we proposed for mtDNA may not be an ideal measure with which to test the uniqueness of subspecies or distinct populations. As found by King et al., if there are slight differences among mtDNA haplotypes, but those haplotypes are fixed or nearly fixed in populations, it will have a substantial effect on the value of Φ_{ST}. That could lead to the erroneous designation of weakly differentiated populations as subspecies or DPS.

We take issue with the frequent use by King et al. of qualitative assessments for F_{ST} and other divergence parameters as being high, moderate, or low. These are subjective assessments that detract from the goal of providing objective tests of genetic uniqueness.

Conclusions

As noted in Ramey at al. (2005) and by at least one peer reviewer of our earlier work (Sites), consistent thresholds for defining conservation units below the level of subspecies have been lacking. However, in proposing thresholds, we also acknowledged that: "*Any such threshold can be seen as arbitrary; however, we hope to establish reasonable threshold levels for these sorts of tests where they have often been absent. Appropriate thresholds can be debated and revised, but we feel that the first step in establishing standards and objectively applying them is to state them explicitly.*" It is legitimate to debate thresholds, but the need for them is obvious -- there are many endangered taxa and not enough resources to conserve them. If conservation effort is allocated to non-distinct or weakly differentiated populations, other more unique taxa lose out. Hypothesis testing relative to these thresholds can provide objective assessments of degree of uniqueness. As legislated in the 1982 amendments to the US-ESA and repeated by numerous authors (cited in Ramey et al. 2005) conservation of endangered taxa would be best served if the allocation of conservation effort were prioritized based on degree of genetic uniqueness.

Literature Cited:

Avise, J. C., and R. M. Ball Jr. (1990) Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Survey of Evolutionary Biology* **7**: 45–67.

Ball, M. R., & Avise, J. C. (1992) Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk* **109**: 626-636.

Crandall.K.A., Bininda-Emonds O.R.P, Mace, G.M., & Wayne R.K. (2000) Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* **15**: 290-295.

Cryan, P. M. (2004) Synthesis of existing information on meadow jumping mice (*Zapus hudsonius*) in the northern Great Plains. An information review compiled for the U.S. Fish and Wildlife Service, Region 6, Denver, Colorado. USGS Fort Collins Science Center, 2150 Centre Ave., Building C, Fort Collins, CO 80526. 27pp.

Cryan, P. M. & Ellison, L. E. (2005) Distributional survey of the meadow jumping mouse (*Zapus hudsonius*) in the northern Great Plains: Trapping Report, Summer 2005. Progress report compiled for U.S. Fish and Wildlife Service, Region 6, Denver, Colorado. United States Geological Survey, Fort Collins Science Center, Fort Collins, CO.

King, T. L., Switzer, J. F., Morrison, C. L., Eackles, M. S., Young, C. C., Lubinski, B., & Cryan, P. (2006) Comprehensive analysis of molecular phylogeographic structure among meadow jumping mice (*Zapus hudsonius*) reveals evolutionarily distinct subspecies. A report submitted to U.S. Fish and Wildlife Service. January 27, 2006.

Jones, G.S. (1981) The systematics and biology of the genus *Zapus* (Mammalia, Rodentia, Zapodidae) unpublished Ph.D. dissertation. Indiana State University, Terre Haute.

Hedrick, P. W. (2001) Conservation genetics: where are we now? *Trends Ecol. Evol.* **16**:629-636.

Krutzsch P. H. (1954) North American jumping mice (genus *Zapus*) *Univ. Kansas Publ. Mus. Nat. Hist.* **4**: 349-472.

Lynch, M. & Crease, T.J. (1986) The analysis of population survey data on human variation. *Mol. Bio. Evol.* **7**: 377-394.

Moritz C (1994a) Defining 'evolutionarily significant units' for conservation. *Trends Ecol. Evol.* **9**: 373-375.

Moritz C (1994b) Applications of mitochondrial DNA analysis in conservation: a critical review. *Molec. Ecol.* **3**: 401-411.

Oyler-McCance, S. J., Kahn, N. W., Burnham, K. P., Braun, C. E., and Quinn, T. W. (1999) A population genetic comparison of large and small-bodied sage grouse in Colorado using microsatellite and mitochondrial DNA markers. *Molecular Ecology* **8**: 1457-1465.

Pergams, O. R. W., Barnes, W. M. & Nyberg, D. (2003) Mammalian microevolution: Rapid change in mouse mitochondrial DNA. *Nature* **423**: 397.

Presa, P., Pardo, B. G., Martínez, P., & Bernatchez, L. (2002) Phylogeographic Congruence Between mtDNA and rDNA ITS Markers in Brown Trout. *Mol. Bio. Evol.* **19**: 2161-2175.

Ramey, R.R., W.M. Boyce, B. Farrell, and S. Kelley. (2000). Phylogeny and host specificity of psoroptic mange mites as indicated by ITS sequence data. *Journal of Medical Entomology* **37**: 791-796.

Ramey R. R. II, Liu, H-P, Epps C. W., Carpenter L M, & Wehausen J. D. 2005. Genetic relatedness of the Preble's meadow jumping (*Zapus hudsonius preblei*) to nearby subspecies of *Z. hudsonius* as inferred from variation in cranial morphology, mitochondrial DNA and microsatellite DNA: implications for taxonomy and conservation. *Animal Cons.* **8**: 329-346.

Riggs, L. A., Dempcy, J. M., & Orrego, C. (1997) Evaluating distinctiveness and evolutionary significance of Preble's meadow jumping mouse: phylogeography of mitochondrial DNA non-coding region variation. Report to the Colorado Division of Wildlife, Denver, Colorado.

Rosenberg, N. A., Mahajan, S., Ramachandran, S., Zhao, C., Pritchard, J. K., & Feldman, M. W. (2005) Clines, clusters, and the effect of study design on the inference of human population structure. *PLOS Genetics* **1**: 660-671.

"Jim Magagna"
<Jim@wysga.org>
05/18/2006 06:25 PM

To <FW6_PMJM@fws.gov>
cc
bcc
Subject FW: Preble's Mouse Comments

The attached comments were sent earlier but returned as undeliverable. This was apparently due to the fact that the printed copy of your March 30 press release had the entire email address underlined making it difficult to recognize that the address included an underscore between the "6" and the "P".

Jim Magagna
Executive Vice President
Wyoming Stock Growers Association
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jim@wysga.org
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-----Original Message-----
From: Jim Magagna
Sent: Thursday, May 18, 2006 4;05 PM
To: 'FW6 PMJM@fws.gov'
Subject: Preble's Mouse Comments

Please see our attached comments.

Jim Magagna
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Mouse Comments May 18.doc

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Region V Vice President- Joe Thomas, *Meeteetse*
Executive Vice President- Jim Magagna, *Cheyenne*

May 18, 2006

Field Supervisor
Colorado Field Office
Ecological Services
USFWS

RE: Preble's Mouse Comments.

Dear Field Supervisor:

The Wyoming Stock Growers Association (WSGA) represents Wyoming landowners engaged in cattle ranching and feeding. Several of our members operate within areas designated as critical habitat for the Preble's Meadow Jumping Mouse. They continue to be significantly impacted by the Service's delay in delisting. Therefore, we appreciate this opportunity to comment on the current status of the delisting process.

It is WSGA's position that the scientific work of Dr. Rob Roy Ramey provided a solid basis for a decision to delist. We were extremely disappointed that the Service chose to solicit additional research from a professional with a reputation for seeking justifications for differentiating "sub-species." However, at this time it is essential to move beyond the work of both Dr. Ramey and Dr. King to resolve the status of the Preble's.

WSGA has, within the past few days, had the opportunity to review the report prepared by Drs. Keith Crandall and Jonathan Marshall of "Genoma" for the State of Wyoming. This report provides a critical scientific analysis of the work of both Dr. Ramey and Dr. King utilizing multiple accepted principles. We urge that this report be given significant weight in the final decision of the Service.

While being critical of the work of both previous researchers, the Genoma report is unambiguous in its conclusions regarding the clustering of multiple subspecies within the areas where the Preble's is currently listed as threatened. According to the authors, "When taken as a whole, the preponderance of evidence and morphometric data indicate that the taxon *Z. h. prebeli* is not a valid taxonomic unit." [Genoma Report, page 28]. In conclusion, the report states "thus using an explicit framework for testing taxonomic boundaries, we find that the "subspecies" *Z. h. preblei* does not even warrant ESU status, let alone subspecific status and therefore recommend it be delisted as an endangered entity under the Endangered Species Act."

WSGA urges the Service to accept this latest scientific research which has independently affirmed the conclusions of Dr. Ramey. It is our expectation that you conclude your review process and make a final decision delisting the Preble's mouse not later that August 6, 2006.

Sincerely,

Jim Magagna

Jim Magagna
Executive Vice President